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HISTOCHEICAL STUDIES ON THE CORNEAL CHANGES INDUCED BY CORNEAL CONTACT LENSES

Junzo HIRANO

Department of Ophthalmology, Nagoya University School of Medicine

In recent years the pervasion of contact lenses is remarkable in our country and it is sure useful to rescue many congenital and aquired eye disorders. However, the cases of corneal injury by the incorrect use of contact lenses also have been reported in no small numbers. Accordingly the fullest knowledge of the ophthalmologist about the contact lens and correct guidance of patients is needed for safe and comfortable wearing of it.

This experiment was made about the safe wearing of the corneal contact lens, used mainly by the author, and at the same time to have full understanding about metabolism and physiology of the cornea.

Experiment I; Relationship between the Wearing Time and Corneal Changes

Method.

A set of corneal contact lenses was prepared the diameters of which were 12, 13 and 14 mm. and the radius of curvature amounted to 7.0 mm. up to 8.2 mm. respectively of each diameters, with a remaining difference of 0.2 mm. each.

The radius of the curvature of the rabbit cornea was measured with Javal-Schiötz ophthalmometer and a contact lens fitted to it was inserted. The nictating membrane was resected prior to the experiment.

The edge of the contact lens, in this case, must not exceed the space width of 1.0 mm. between the corneal limbus.

The eyes were enucleated after the wearing of 3,6,12,24,48 hours and 10 days, and fixed in pure alcohol, then the paraffin sections of 10μ in thickness were made. These sections were stained by hematoxylin-eoisn staining, methyl green-pyronin method, Feulgen reaction for DNA and Ninhydrin-Schiff reaction for amino acid.

In this experiment five eyes were employed to each wearing time and corneal changes were taken of the average of each. On the other hand, upper and lower lids of the rabbit were sutured together to interfere with atmospheric oxygen in place of inserting contact lens, and the cornea was taken as the control.

Results.

Ocular symptom and histological views. About six hours after the insertion, the increase of eye discharge and injection of bulbar conjunctiva were observed. These changes have grown gradually and there appeared also ciliary injection after 12 hours.

Histologically, dilation of the limbal cappllaries was observed corresponding to ocular symptom, but the cornea was as yet intact.

After 24 hours characteristic lustur of the corneal surface was lost, and in some cases a slight haze was observed.

In histological view, widening of the intercelluiar spaces of the corneal epithelium and the disarrangement of the epithelial cells were observed in addition to the dilation of iris vessels and slight thickening of the corneal stroma. (Fig. 1-4)

Fig. 1—4. Various stages of rabbit cornea following application of corneal contact lens. Stained with hematoxylin and eosin.

Fig. 1. normal cornea

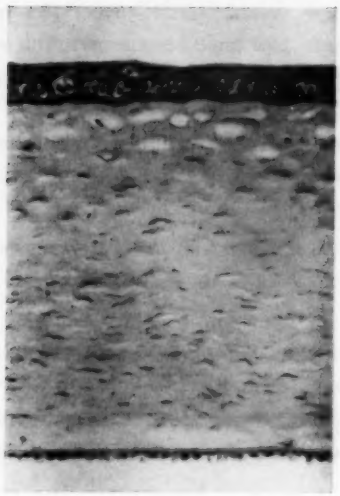


Fig. 2. 24 hours

Fig. 3. 48 hours

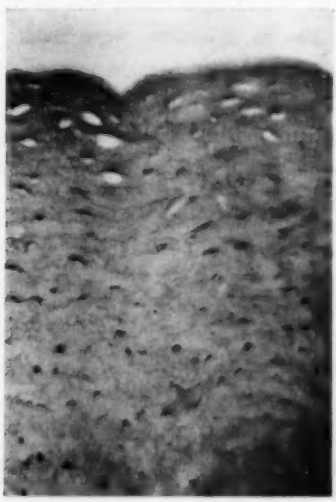
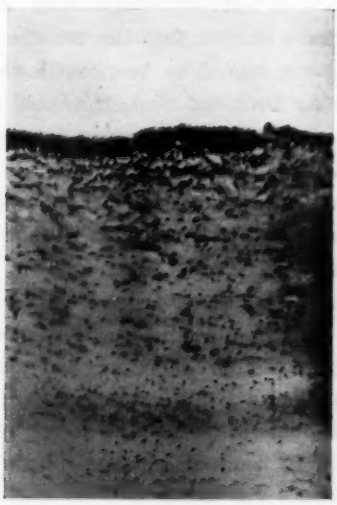


Fig. 4. 10 days



Changes of glycogen. The most remarkable and characteristic were the changes seen in corneal glycogen. In normal rabbit eye a small mass of glycogen strongly positive to PAS staining arranges itself one by one at the bottom of the basal cell in the corneal epithelium just as a layer of brick paving on the basement membrane. In the middle and superficial layers of the cornea glycogen maldistributes itself at the periphery of the cells or intercellular spaces.

Thus the corneal epithelium contains glycogen in high concentration, however, it disappears completely by the salivary test. This indicates that the glycogen in the cornea is low in polymerization and so ready to convert into energy.

Three hours after the insertion a diminution in size and concentration of the glycogen masses was observed at the bottom of the basal cells in the central region of the cornea, but no changes in the upper layers as yet.

After 6 hours the glycogen mass has been stained faintly, smaller in size, and obscured. Although glycogen was still observed in the upper layers, the maldistribution to the periphery of the cell and intercellular space was lost, the contours of cells fading into a pink zone.

Twelve hours afterwards no trace of glycogen masses seen on the basement membrane, and only in the superficial layers weakly positive PAS reaction could be observed. Glycogen disappeared completely from the whole epithelium after 24 hours.

In the peripheral zone of the cornea, not covered by the corneal lens, glycogen still stained normal after 24 hours, and though a desquamation of the corneal epithelium occurs in the central region, glycogen still remained in the survinig epithelial cells after 48 hours.

Except for the epithelium, no effect on PAS staining due to corneal contact lens was observed in the cornea. (Fig. 5—10)

Fig. 5—10. Various stages of rabbit cornea follwing application of corneal lens. PAS stain. Fig. 5—9 show the center, and Fig. 10 the limbus of the cornea.

Fig. 5. normal cornea

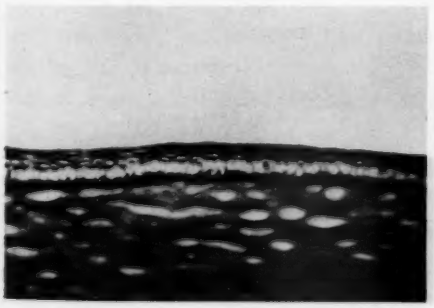


Fig. 6. 3 hours

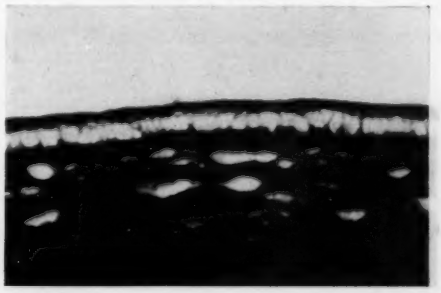


Fig. 7. 6 hours

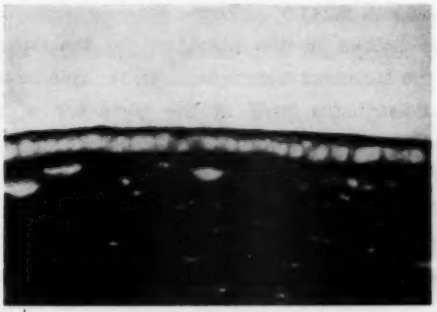


Fig. 8. 12 hours

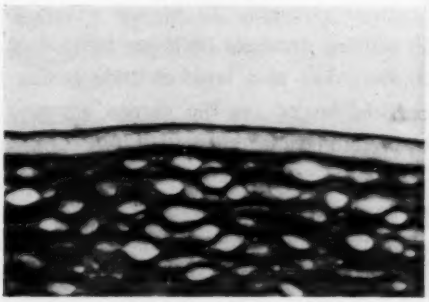


Fig. 9. 24 hours

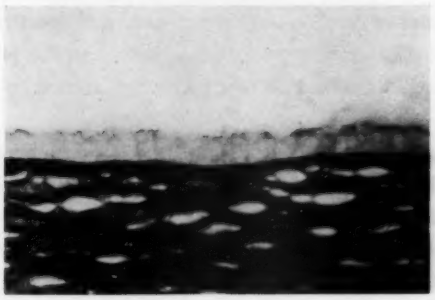
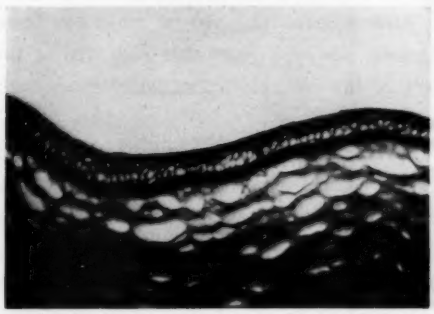


Fig. 10. 24 hours



Changes of nucleic acid and amino acid. Changes of these substances are by far slower compared with glycogen. No changes occured after the insertion of 12 hours. Twenty-four hours after low staining with pyronin and weakening of Ninhydrin-Schiff reaction appeared in the protoplasma of the epithelial cell, but no changes of methyl green staining, Feulgen reaction, Ninhydrin-Schiff reaction were seen in the nuclei. These changes were marked especially in the center of the cornea. (Fig. 11—14)

Fig. 11—14.

Fig. 11. normal cornea
methyl green-pyronin method

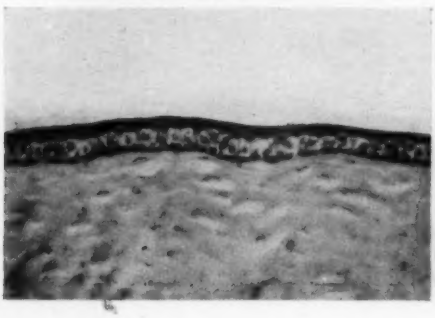


Fig. 12. 24 hours application of contact lens
methyl green-hyronin method

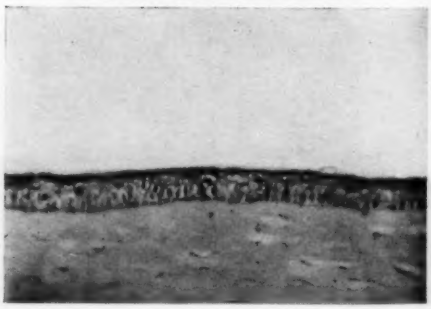


Fig. 13. normal cornea
Ninhydrin- Schiff method

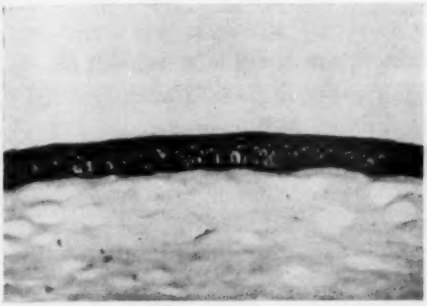
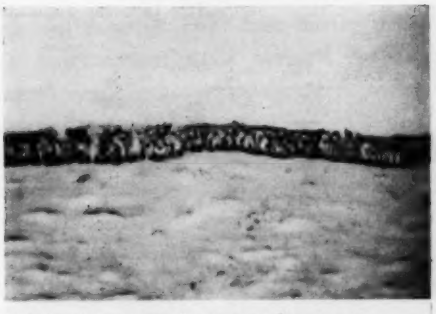


Fig. 14. 24 hours
Ninhydrin-Schiff method



Control. Corneas of the rabbits which completely intercepted the atmospheric oxygen by a compact suture of upper and lower lids for 48 hours showed no histological and histochemical changes.

Experiment II: Recovery Process

Method.

Following the insertion of the corneal lenses for 12 hours they were removed and then the rabbit eyes were enucleated after the lapse of 1,3,6,9,12 and 18 hours. These materials were stained by PAS method.

Results.

One hour afterwerds: Equal to the case of 12 hours insertion, no resynthesis of glycogen in the corneal epithelium was observed.

Three hours afterwards: A fine PAS positive zone has appeared on the basement membrane growing to the glycogen mass hereafter. The increrse of glycogen was observed diffusely in the epithelial cells of the middle and superficial layers i.e. the resynthesis of glycogen has been carried out to the extent barely detectable with PAS staining at this time.

Six hours afterwards: Glycogen has come to stain deeper at the bottom of the basal cells and also in the upper layers, but the contour of each cell was not yet distinct.

Nine hours afterwards: Glycogen in the upper layers has come to take a normal aspect maldistributing to the periphery of the epithelial cells and intercellular space. Glycogen mass in basal layer also has come to react distinctly to PAS staining but still remained small and flat compared with normal aspect. Namely the corneal epithelium is still on the way to recovery.

Twelve hours afterwards: Glycogen in the epithelium has been resynthesized almost equal to the normal cornea.

This result indicates that the influence of corneal lens on the corneal epithelium disappeares after 12 hours in the case of 12 hours wearing.

Ocular signs such as conjunctival injection, discharge, ciliary flush etc. caused by wearing of contact lens disappeared after 3 hours of its removal, which are more rapid than the reappearence of glycogen in the epithelium. (Fig. 15-19)

Fig. 15—19. Various recovering stages of glycogen in the corneal epithelium following removal of corneal contact lens after 12 hours application. Stained by PAS method.

Fig. 15. 1 hour

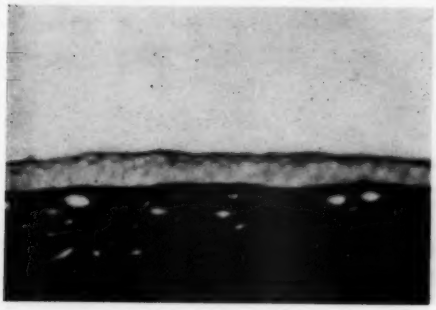


Fig. 16. 3 hours

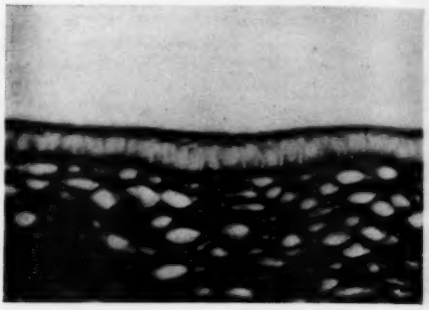


Fig. 17. 6 hours

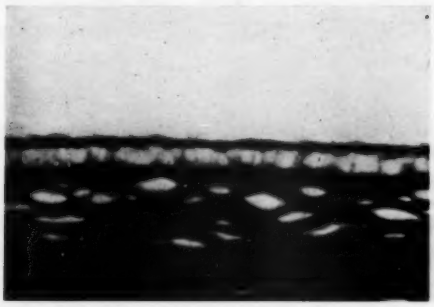


Fig. 18. 9 hours

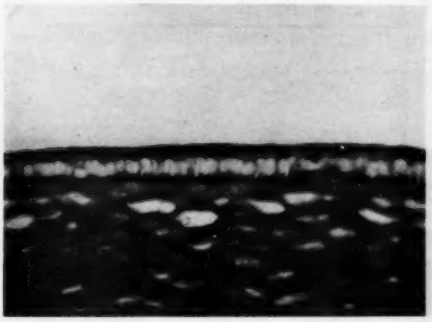
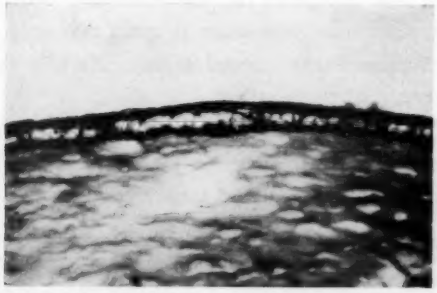


Fig. 19. 12 hours



Experiment III: Effect of Habitual Wearing

Methods.

The right eyes of rabbits were worn with corneal lenses 3 hours daily during the first week and the time of wearing was prolonged one hour weekly, while the left eyes were left free of corneal lenses. At the end of the fourth week both eyes were worn with the corneal lens for six hours then enucleated both eyes and examined with PAS method.

Another group of the rabbits differed on the wearing time of the first week, the prolongation time of the following weeks and the wearing time of both eyes after the end of the fifth week, respectively 4 hours, 2 hours and 12 hours.

Results.

The results of PAS staining have shown no difference between the right and left eyes, namely the effect of habitual wearing was not recognized in all cases of the experiments.

Discussion

Respiration of the cornea is fairly active and oxygen intake is done through the corneal surface. Limbal plexus and aqueous humor are, however, thought to be of no importance as the routes of oxygen supply to the cornea[1),3),5)].

Langham[6)] and Smelser[10),11)] have proved, that oxygen diffuse into the precorneal tear film from the surrounding atmosphere, when the lids are open and also from the vascular bed of palpebral mucosa when the lids closed. The cornea will fall into anoxia when contact lens is worn, because of the depriving of oxygen supply mentioned above.

It is the well-known fact that in anerobic glycolysis twenty fold carbohydrate is required to yield as much energy as in the process of complete oxidation. Thus in cornea without oxygen supply, carbohydrate consumption takes place so rapidly that glycogen disappeares from the epithelium. In similar experimental condition Smelser[11)] found lactic acid accumulation in the corneal tissue and proved the increased anerobic glycolysis.

While PAS positive substances in the stroma are compound polssaccharides being the tissue component and not the energy source of the corneal metabolism, they showed no changes throughout the auther's experiment.

The disappearance of glycogen, which is the energy source of living cells must have some effect on the other metabolic systems of the cornea. Especially RNA of the cornea tends to vary parallel to glycogen in many environmental conditions, and as the distribution of RNA in the corneal epithelium, also, is similar to that of glycogen, it is natural that RNA decreases successively with the disappearance of glycogen, when the contact lens is inserted. On the contrary DNA is essentially stable and not to be affected by any nutritional condition of cells.

Glycogen existing abundantly in the corneal epithelium is considered as the energy source to regulate the hydration of the cornea. Accordingly exhaustion of glycogen causes an abnormal hydration of the cornea. Thus the optical property is lost and then cornea becomes hazy.

In the experiments of many authors[2),4),8),9),11)] up to the present, contact lenses used were all of scleral corneal type. Result of this experiment using corneal lens shows, that the corneal injury is far slighter compraed with that of the scleral corneal lens.

This reason is explained firstly by an existence of a limbal region being exposed in the case of corneal lens which permits free corneal respiration. Secondly as the corneal lens remained only on the cornea, not extending over the limbus, ilmbal circuration cannot be strangulated. Furthermore, as corneal type needs no contact lens solution, the cornea is saved from its ill effect.

Conclusions

1. Histochemical changes of the rabbit cornea were investigated when corneal contact lens was applied on it.

The most remarkable changes were recognized in the distribution of glycogen in the corneal epithelium. Slight decrease in epithelial glycogen could be seen 3 hours after the insertion and complete disappearance after 24 hours. In the limbal region, however, it remained normal at 24 hours after the insertion.

2. RNA and amino acid in the protoplasma of the epithelial cells decreased gradually after the disappearance of glycogen, but DNA in the nucleus was always intact.

3. Histologically the disarrangement of epithelial cells and the slight thickening of the corneal stroma were observed after 24 hours, and desquamation of epithelium, opacity and thickening of the stroma after 48 hours was evident.

4. These changes were especially marked in the central region of the cornea.

5. The recovery process of the cornea was observed after previous insertion of the corneal contact lens for 12 hours. The resynthesis of epithelial glycogen has been seen after 3 hours and it was completed after 12 hours.

6. No effect of habitual wearing for five weeks was observed histologically.

7. The injury of the cornea by the insertion of the corneal contact lens was recognized as far slighter compared with the scleralcorneal type.

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ELECTROMYOGRAPHIC STUDIES ON THE MECHANISM OF LIMITATION OF MONOCULAR MOVEMENT

Hirofumi MOMOSSE

Department of Ophthalmology, School of Medicine, University of Tokyo

The globe keeps on rotating, so far as the tension of the contracting muscle is surpassing that of the relaxing muscle. The rotation of the globe ceases at the point where the tension of the contracting muscle reaches its maximum and yet can no longer surpass that of the relaxing muscle. That position of gaze is the limit of the field of monocular fixation. However, our present knowledge concerning the mechanism of limitation of the field of monocular fixation is not yet based upon any innervational proof.

The electromyographic studies on the innervation to each extraocular muscle in monocular movements was reported previously[1]. In the present work, an attempt was made to investigate electromyographically the quantitative changes of innervation to the contracting and the relaxing muscles, especially at the limit of the field of monocular fixation.

Materials and Methods

Observations were made on about 10 volunteers, all around 20 years of age, with normal ocular motility and good vision.

Bipolar needle electrodes with the bared tip of 2 mm. were employed. It was confirmed, by the preliminary experiment, that the uncoated tip of 1~1.5 mm. was enough to reveal the electrical behaviors which may represent the whole electrical activity of the muscle.

Action potentials induced from the individual muscle were led to an electromyograph with push-pull type amplifier. Moreover, one of the outputs was directly, and the other was through the integrator, led to an oscillograph of electromagnetic type; direct EMG wave and integrated EMG curve were simultaneously recorded on a moving oscillograph-paper.

The fixation target was moved along the spherical surface of the perimeter of 60 cm. in diameter. The non-tested eye was covered during the experiment.

Details were stated in the previous report.

Results and Discussion

As the fixation line was moved along the horizontal cardinal plane through the primary position, the action potentials of the medial rectus changed as shown in fig. 1. The integrated EMG curve on contraction (shortening curve) ascends, retaining a concave tendency and forms a peak just at the limit of the field of fixation. Despite the strain to continue further rotation, the action potentials increase no longer, but rather decrease; that is, the impulse to the contracting muscle increases and eventually reaches the maximum at the limit of the field of monocular fixation. The integrated EMG curve on relaxation (lengthening curve) descends along nearly the reverse course, and at the outward

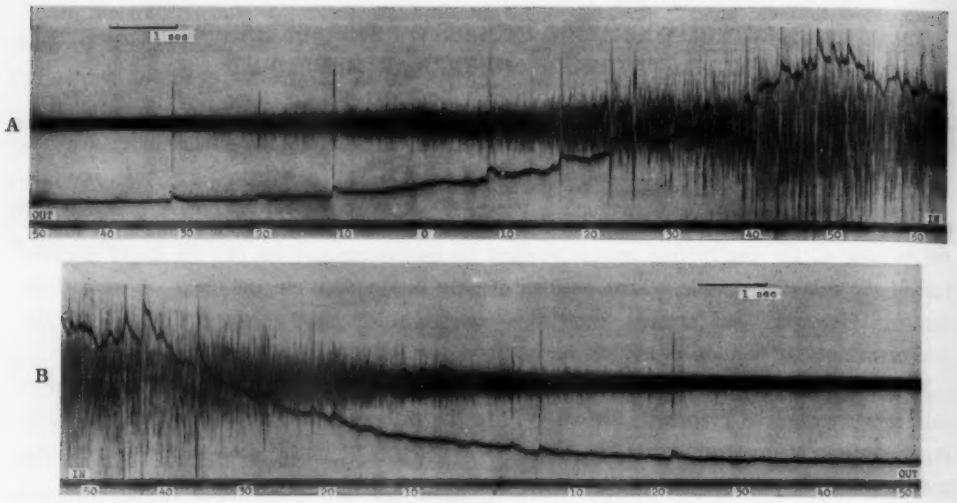

Fig. 1. Horizontal movement. Medial rectus.

A. Integrated EMG curve (shortening curve) and direct EMG wave by contraction.

B. Inetgrated EMG curve (lengthening curve) and direct EMG wave by relaxation.

limit the impulse to the relaxing muscle reaches the minimum, approximately zero.

The action potentials of the medial rectus in oblique movement through the primary position are shown in fig. 3 above. The shortening curve is almost the same as that in horizontal movement (fiig. 3 below) in shape, but in height, there exists a difference between the two, that is, the peak of the former is lower than the latter.

Behavior of the lateral rectus was just the same as the medial rectus. Behavior of the inferior rectus and the inferior oblique in the vertical cardinal movement was similar to that of the horizontal recti in the horizontal movement; in the oblique movement the behavior of them was the same as that of the horizontal recti.

It has been hitherto considered that the extraocular muscles are a typical isotonic contractor. The first of the reason is, that the movement of the globe is similar to that of the fixed pulley from the viewpoint of the anatomic structure of the globe-orbit joint and the acting direction of the extraocular muscles; the second, the eye movement is not accompanied with the antigravitional work, unlike the muscles of the extremities.

However, the present experiment revealed, that the contraction and relaxation of the extraocular muscles are not purely isotonic. The reasons are as follows:

In order that the eye starts its movement outward from the primary position, the impulses to the lateral rectus must increase slightly with a slight decrease in the impulses to the medial rectus, resulting in a slight disparity between the tensions of the two. In general, the tension of the muscle is in parallel relation to (not in proportion to) the muscle length, if the action potentials are constant; therefore, the action potentials must

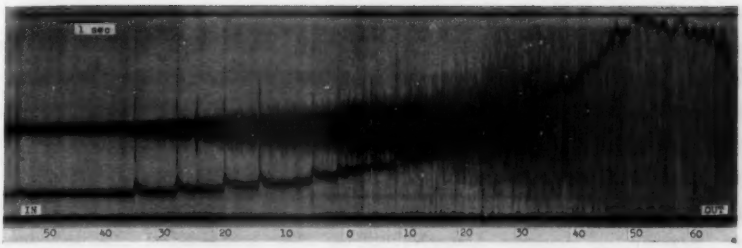

Fig. 2. Horizontal movement from 50° (in) to 60° (out). Lateral rectus.

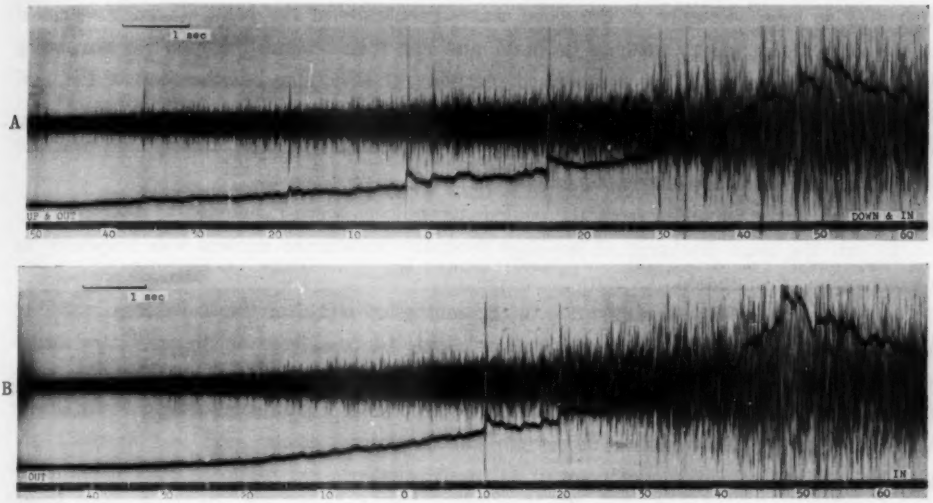

Fig. 3. Medial rectus.
A. Oblique movement through the primary position.
B. Horizontal movement from 50° (out) to 60° (in).

keep on increasing in order to maintain the same tension during the shortening of the muscle. Accordingly, by abduction, the action potentials of the lateral rectus must keep on increasing to contract isotonically. In a similar way, the impulses to the medial rectus must decrease gradually during the lengthening to maintain the same tension.

Thus, maintaining a slight difference between the tensions, the contracting and the relaxing muscles rotate the globe with isotonic contraction and relaxation. When the globe moves outward beyond a 20°~30° position, however, the lengthening curve begins to show the horizontality. Since the action potentials of the relaxing muscle decrease no more in spite of the constant lengthening velocity of the relaxing muscle, the tension is caused to increase gradually. On the other hand, in order to keep on rotating the globe, the tension of the contracting muscle should increase accompanied with an accelerative augmentation of the discharge. The shortening curve of the contracting muscle begins to reveal a steep ascendence at the position of 20°~30°, as shown in fig. 1,2 and 3.

In general, the relation between the shortened length and the increased part of the action potentials is still unknown in the case of isotonic contraction. It is therefore impossible, in ocular movement, to deduce the far advanced conclusion concerning the change of muscle tensions from the present result. However, it is evident, that the tensions of the two muscles increase beyond a 20°～30° position.

The tension of the contracting muscle, though it is increasing, cannot keep on surpassing the augmenting counter power (that is, the synthesized reaction including the passive pull of the lengthening and therefore tightening muscle and the check ligament etc.) ever so long. Rotation of the globe ceases eventually at the point where the tension of the contracting muscle reaches its maximum and then becomes incapable of surpassing the augmenting tension of the synthesized reaction. It is nothing but the limit of the field of monocular fixation.

From the fact that the action potentials of the contracting muscle reach the peak-like maximum, the following inferences may be drawn:

1) The entire impulses from the oculomotor nulceus were exhausted to discharge;

2) or, the more discharge of impulses from the oculomotor nucleus was suspended by a mechanism of inhibition.

The afferent sensory impulses due to the contracted strain of the shortening muscle or due to the stretch of the lengthening muscle may be considered as the inhibitory factors in 2); the afferent impulses, however, are not necessarily restricted to that from the extraocular muscles only. Furthermore, the hypothesis of an innervation sense (Irvine & Ludvigh, 1936[2]) also must be taken into consideration.

Considering from the standpoint of 1), some questions are raised in the oblique movement. The action potentials of the lateral rectus at the up- and out-ward limit of the field of fixation are less in quantity, than those at the outward limit of the horizontal cardinal movement, and the action potentials of the superior rectus and the inferior oblique at the up- and out-ward limit are less than those at the upward limit of the vertical cardinal movement respectively. It is difficult to explain why the field of fixation is limited without the maximum inflow of impulses to the three muscles, despite the demand from the motor cortex, i.e., the conscious effort to rotate the globe still more.

Adopting assumption 2), it may be explained as follows. The inhibitory factor for each one of the nuclei of the three muscles does not amount to the sufficient quantity, but the sum of them does, so that three oculomotor nuclei are all equally inhibited.

Summary

In the horizontal movement of the eye from the primary position, the horizontal recti reveal an isotonic contraction and relaxation, maintaining a slight difference of tensions. When the eye rotates beyond a 20°～30° position, however, the tension of the relaxing muscle begins to increase gradually, therefore that of the contracting muscle should also increase to maintain the rotation of the eye; i.e., the isotonic contraction and relaxation

begin to be accompanied with a factor of isometric contraction and relaxation.

The impulses to the contracting muscle reach the maximum just at the limit of the field of monocular fixation, in both the cardinal and the oblique movements. Concerning the fact that three contracting muscles reveal less quantity of action potentials at the limit of the field of fixation in the oblique movement than in the cardinal movement, the existence of the inhibitory mechanisms for the oculomotor nuclei was discussed.

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STUDIES ON THE ARTERIAL SUPPLY OF THE VISUAL CORTEX AND THE OTHER OCCIPITAL REGION IN MAN

Masaaki SHINOHARA

Departments of Ophthalmology and Anatomy, Faculty of Medicine, Kyushu University

The arterial supply of the occipital lobe has already been studied by Duret, Beevor, Shellshear, Beauvieux and Ristich-Goelmino, and Itahashi, but the arterial supply of the sulci and gyri of this region, especially on the outer surface, has not been completely described yet. Moreover, within my knowledge, except for Shellshear's incomplete study, no research has been made on the arterial supply of the striate area. For this study, thirty-three brain-hemispheres of Japanese adults were used, all but two of which had been formalin-fixed and a five to ten % gelatine solution tinged with India ink was injected successively by hand into each branch of the three main cerebral cortical arteries using an injector with a blunt needle. After observation of the arterial supply, one mm. sections were made by hand from the region of the occipital pole and two to three mm. sections from the other region. By this means the striate area was revealed.

1). Sulci and Gyri in the occipital lobe

Sulcus retrocalcarinus (S.RCAL.): In 16 of the 33 hemispheres, it was situated vertically on the posterior border of the mesial surface or a little more anteriorly. Its relation to the occipital pole in the other cases was as follows: in 5 hemispheres it reached the occipital pole, in 4 hemispheres it approached it, and in the remaining 8 hemispheres, it passed to the outer surface through the occipital pole. Sulcus infrastriatus (S.IST.): It was situated at the point where the mesial surface met the tentorial surface of the occipital lobe. It was found in 26 hemispheres, forming an operculum in 16 of 24 hemispheres and demalcating the striate area inferiorly or anteriorly-inferiorly. Its greatest depth was 6-14 mm.. In 15 out of 26 hemispheres, the striate area entered this furrow from the upper edge partly or along the whole length for 1-4 mm.. Sulcus lunatus (S.LUN.): Three types were distinguished. The first type, observed in 25 hemispheres,

had such a form as embraced the occipital pole and consisted of one sulcus in 5 hemispheres, two sulci in 12 hs., three in 7 hs. and four in 1 h.. The second type, observed in 5 hemispheres, consisted of a superior and an inferior furrow. The latter tended to be better developed than the former and to pass forwards-upwards. The third type, which was observed in 3 hemispheres, consisted of one furrow lying almost horizontally, which was the combination of a S.LUN. and a sulcus praelunatus (S.PL.).

The author termed the area, which was surrounded by the S.LUN. and the vertical S.RCAL., or surrounded by the S.LUN. and the border of the hemisphere when the vertical S.RCAL. was absent, "gyrus poli occipitalis (G.P.O.)". The outer surface of the occipital lobe outside of the G.P.O. was divided by either the S.PL. or the S.LUN. of the third type into gyrus occipitalis superior (G.O.S.) and gyrus occipitalis inferior (G.O.I.). The former was subdivided into three parts, A,B, and C. The area A was surrounded by incisura sulci parietooccipitalis (I.S.PO.), sulcus interparietalis (S.IP.), sulcus paramesialis (S.PM.) and sulcus occipitalis transversus (S.O.T.). The area B was surrounded by S.PM., S.LUN., S.O.T. and S.PL.. But sometimes according to the location of the S.O.T., S.PM. and S.PL. did not form the border of the area A and B. The area C was the other region of the gyrus occipitalis superior.

2). The extension of the striate area

In the inside of the hemisphere: The striate area, whose anterior extremity was found at various points of the bottom of truncus sulci calcarini (T.S.CAL.), extended backwards only on its inferior surface except in the case of 11 hemispheres in which gyrus cunei of Ecker was present and the striate area extended also on its superior surface over a narrow width from its posterior bottom. On the gyrus lingualis (G.L.), in 24 hemispheres, the striate area appeared behind the middle point of the T.S.CAL., in 26 hemispheres, it was limited by the upper lip of the S.IST. inferiorly or anteriorly-inferiorly and in 7 hemispheres, it extended over one-fourth to three-fifth of the mesial surface. In the S.CAL. it extended over the whole area in 16 hemispheres and over the whole area except for one part of the medial-anterior region of the superior surface in 17 hemispheres. In the S.RCAL. it extended over the whole or nearly the whole surface, with the exception of one hemisphere in which it extended over the greater part. On the cuneus it extended over varying dimensions of the posterior, the inferior or the posterior-inferior section in all cases, except, in the case of one hemisphere in which it extended over only the middle part.

In the gyrus poli occipitalis: In the cases which had the first type of S.LUN., the striate area extended over all or the greater part of the gyrus poli occipitalis in 4 hemispheres, over two-thirds or less of the gyrus poli occipitalis in the medial section in 11 hemispheres, over one-third to two-thirds of it in the inferior-medial section in 4 hemispheres, over three-fifths of it in the superior-medial in one hemisphere and over one-third to a half of it concentrically with S.LUN. in 5 hemispheres. In the cases which had the second type of S.LUN., the lateral border of this area was not so clearly defined. The

striate area extended over a limited area of the medial or the medial-inferior section. In the cases which had the third type of S.LUN., the posterior extremity of the striate area corresponded to the occipital pole or touched it, and the posterior region of the striate area extended over a limited area along the vertical S.RCAL., when it was present. In the cases which had the first and the second types of S.LUN., the striate area extended with a narrow width along the border of the hemisphere to either or both of the gyrus occipitalis superior and inferior in 9 hemispheres. In only 3 hemispheres the striate area was limited laterally by the caudal lip of the S.LUN..

The posterior extremity of the striate area touched the occipital pole in 3 hemispheres and corresponded to it in 6 hemispheres. The posterior region of the striate area extended to the outer surface including the occipital pole in 24 hemispheres. Measuring from the mesial border of the hemisphere, the distance varied from 0.5-1.0 cm in 8 hemispheres, 1.2-2.0 cm in 11 hemispheres to 2.2-3.0 cm in 5 hemispheres.

3). The cortical branches of the posterior cerebral artery (A.C.P.) and their distributions

The author classified the cortical branches of the A.C.P. into arteria occipitotemporalis (A.OT.) and arteria occipitalis (A.OC.). The former was mainly distributed to gyrus hippocampi, gyrus occipitotemporalis medialis (gyrus lingualis), gyrus occipitotemporalis lateralis (gyrus fusiformis), sulcus temporalis inferior, sulcus occipitalis inferior, S.RCAL. and gyrus poli occipitalis; the latter, mainly to T.S.CAL., S.CAL., S.PO., S.PM., cuneus, the posterior part of praecuneus and gyrus occipitalis superior A. Arteria occipitalis was subdivided into arteria parietooccipitalis (A.PO.) and arteria calcarina (A.CAL.) at various points in the T.S.CAL.. Its separating point was situated behind the middle point of T.S.CAL. in 20 hemispheres. The A.CAL. was not found in 5 hemispheres; in 4 hs. S.CAL. was wholly supplied by the A.OT. and in 1 h. it was supplied by A.OT. and A.PO.. A.PO. developed better than A.CAL. in 21 hemispheres and equally to A.CAL. in 5 hs.. A.CAL. developed better than A.PO. in only 2 hemispheres. A.PO. ramified a small branch to S.CAL. in 7 hemispheres and A.CAL. to S.PO. in 4 hs.. There existed one A.OT. in 10 hemispheres, two in 14 hs., three in 8 hs. and four in 1 h..

On the tentorial surface of gyrus lingualis, A.OT. was distributed to the whole or the greater part in almost all cases. S.PO. was supplied mainly by A.PO., but sometimes either or both of arteria cerebralis anterior (A.C.A.) and arteria cerebralis media (A.C.M.) supplied some areas of the superior or anterior-superior part. Cuneus was suppiled by one, two or all of the A.CAL., A.PO. and A.OT.. Each of the former two had about the same area to supply. The last had the smallest area to supply. A.PO. was distributed to the anterior or anterior-superior part. S.PM. was supplied by one, two or three of A.PO., A.C.M., A.CAL. and A.OT.. The first was the most dominant of all. A.C.M. and A.CAL. were next in dominance and each had the same area to supply. Gyrus poli occipitalis was supplied mainly by A.OT. and A.C.M.. The former was considerably more dominant than the latter. Into this area entered A.C.M. from the upper, the lateral or the upper-lateral side, A.CAL. from the medial or the upper-medial and rarely A.PO. from the upper or

the medial-upper. Gyrus occipitalis superior A was supplied by A.PO. and A.C.M.. The former was considerably more dominant than the latter which was distributed backwards or outwards-backwards. Gyrus occipitalis superior B was supplied mainly by A.C.M.. Sometimes A.PO. was distributed inwards or inwards-upwards to some extent. A.OT. or A.CAL. rarely entered this area from the medial. Gyrus occipitalis superior C was supplied only by A.C.M.. S.LUN. was supplied mainly by A.C.M. and A.OT.. The former was considerably more dominant and entered this furrow from the lateral or the lateral-superior. A.CAL. or A.PO. rarely entered a part of this furrow from the superior or the medial-superior. S.IP. in the occipital lobe was mainly supplied by A.C.M.. Sometimes A.PO. entered from the medial. The inner limb of S.O.T. was supplied by A.C.M. and A.PO., and each had nearly the same area to supply. The outer limb of S.O.T. was mainly supplied by A.C.M. but in 2 hemispheres A.PO. supplied the whole area. S.PL. was supplied mainly by A.C.M. but in some cases A.OT. entered from the posterior. Gyrus occipitalis inferior was supplied by A.C.M. and A.OT.. The former was a little more dominant in supplying and was distributed to the superior or the superior-anterior part. S. occipitalis inferior (S.O.I.) was supplied mainly by A.OT., but in some cases A.C.M. entered the superior (or lateral) surface.

4.) *The arterial supply of the striate area*

The striate area in the T.S.CAL. was supplied by one, two or three of A.CAL., A.OT., the trunk of A.OC., and A.PO., but this area was rarely supplied by three arteries. Each of the former two had the same area to supply and was considerably more dominant than the others. The striate area in the S.CAL. was supplied by one, or two, or all of A.CAL., A.OT. and A.PO.. The first was the most dominant, and the second was next in dominance. A.PO. was rarely distributed upwards. A.OT. was distributed downwards to backwards. In most cases it was supplied by two arteries. Although in 12 hemispheres it was supplied by only one artery, that is in 8 hs. by only A.CAL., and in 4 hs. by only A.OT.. It was rarely supplied by three arteries. The striate area in the S.RCAL. was supplied mainly by either A.OT. or A.CAL. or both. The former was conspicuously more dominant and was distributed downwards-backwards. A.C.M. entered a part of this area posteriorly in 4 hemispheres. The striate area in gyrus lingualis was supplied mainly by A.OT.. A.CAL. was distributed from the anterior-superior or superior edge to the whole area in 4 hemispheres, to one-third to a half of this area in 3 hs. and to only a part in 9 hs.. The striate area in cuneus was supplied mainly by A.CAL. or A.OT.. The former was a little more dominant than the latter. Sometimes A.PO. supplied some area of the anterior or the anterior-superior region. The striate area in gyrus poli occipitalis was supplied by one, two or three of A.OT., A.C.M., A.CAL. and A.PO.. The first was definitely more dominant than others; A.C.M. and A.CAL. were next in dominance and each had the same area to supply. A.PO. rarely entered the uppermost part of this area. The striate area in this region was supplied by only one artery, A.OT., in 8 hemispheres, by two in 15 hs. and by three in 10 hs..

A.CAL. entered this region from the medial or the medial-superior border. The branches of A.C.M. entered this region from the lateral or the superior-lateral border in 60.6% of the cases and reached as far as the 0.5 cm border region in 30.3% of the cases.

5). *Arterial anastomoses in the pia mater*

Anastomoses between A.C.P. and A.C.A., or A.C.M.: There were some main anastomotic branches with the calibers of 0.1-0.3 mm., but rarely 0.4-0.7 mm.. Between these main anastomotic branches, there was a greater number of finer ramified anastomotic branches with the calibers of 0.03-0.05 mm.. Either because the materials were not good or because the injections were not properly made, the anastomoses could not be observed in the following cases: between A.C.P. and A.C.A. in 11 hemispheres; between A.C.P. and A.C.M., on the mesial border of the hemisphere or the neighbouring outer surface in 15 hs., on the lateral border of the occipital lobe in 21 hs,, and on the area around the occipital pole in 19 hs..

Anastomoses between branches of A.C.P.: Generally finely ramified branches formed an anastomotic network. The calibers of these anastomoses which could be observed in 8 hemispheres were 0.01-0.05 mm.. From four to twelve anastomoses were found in a square cm.. In 6 hemispheres a few anastomoses with calibers of 0.1-0.2 mm. were found.

Summary

The author found an anastomotic network in each system of the three chief cerebral cortical arteries, which had not been found by Duret and Beevor, but which had been found by Beauvieux and Ristich-Goelmino, and by measuring the calibers of the anastomoses, the author proved that the anastomoses between the different systems were larger than those within the same system. In 27 of 33 hemispheres, the author observed such cases as had been stated by Beevor, that is in nearly half the cases the posterior limit of the A.C.M. extended to the occipital pole, or a half in. anterior to it. Beauvieux and Ristich-Goelmino found no A.C.M. reaching the occipital pole. In the author's specimens, in 15 hemispheres the occipital pole was supplied by A.OT., in 4 hs. by A.C.M., in 1 h. by A.CAL., in 8 hs. by A.OT. and A.CAL., in 4 hs. by A.OT. and A.C.M., and in 1 h. by A.CAL. and A.C.M.. Anastomoses between the arteries converging to the occipital pole were observed in some of the cases. Shellshear stated that the distribution of the anterior, the middle, the posterior temporal and the calcarine artery closely corresponded to the area 20, 37, 18 and 19, and 17 of Brodmann. But as a general rule, such relations as mentioned above were not observed in the author's specimens.

The striate area was mainly supplied by the calcarine and the occipitotemporal artery, and each had about the same area to supply. The latter was distributed downwards to backwards. In some cases, some part of the anterior or the anterior-superior region was supplied by the parietooccipital artery, and an anterior part by branches of the trunk of the occipital artery. The branches of the middle cerebral artery entered the striate area in the gyrus poli occipitalis in 61% of the specimens and reached as far as the 0.5 cm border region in 30% of the cases.

The striate area in the gyrus poli occipitalis is the region in which the branches of the middle cerebral, the occipitotemporal, the calcarine but rarely the parietooccipital artery converge. For this reason it is not easily affected by the various vascular lesions of the occipital lobe.

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Table 1. The arterial supply of the striate area

Striate area / Arteries	in T.S. CAL.				in S. CAL.				in S. RCAL.				in G.L.				in CUNEUS				in G.P.O.			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
trunk of A. OC.	3	1	1					1								2								
A. CAL.	11	5	3		17	3	3	5	7	4	1	3	4		3	9	18	2		3	3		1	10
A. PO.		3	3			1	2	4									1	3		7				3
A. OT.	11	5	1		8	3	2	9	19	4	2		26	3			11		1	1	24	1	1	4
A. C.M.												4								1	3	1		16

$A \geqq {}^3/_4$, $^3/_4 > B \geqq {}^1/_2$, $^1/_2 > C \geqq {}^1/_4$, $D > {}^1/_4$

Numbers indicate the number of brain-hemisphere.

The explanation of figures 1—5

The shadowed region is the striate area.

Arteries

□: A.C.A., △: A.CAL. (C), ×: A.PO. (P), ○: A.OT. (O), Blank space in the back views: A.C.M. (M)

Sulci cerebrales

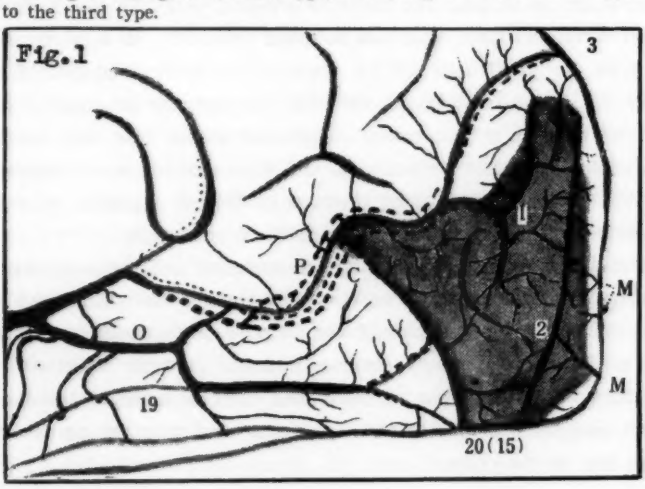
1. S.CAL., 2. S.RCAL., 3. S.PO., 4. S.PM., 5. S.PARIETALIS TRANSVERSUS POSTERIOR, 6. S.IP., 7. S.O.T., 8. S.LUN., 9. S.PL., 10. S.O.S., 11. S.O.I., 12. S. OCCIPITALIS ANTERIOR, 13. RAMUS ASCENDENS SULCI TEMP. SUP., 14. S. TEMP. MEDIUS, 15. S. OCCIPITOMARGINALIS BASIALIS of HISHIDA, 16. S. SAGITTALIS CUNEI SUP., 17. S. SAGITTALIS GYRI FUSIFORMIS, 18. T.S.CAL., 19. S. COLLATERALIS, 20. S.IST., 21. S. SAGITTALIS CUNEI INF., 22. FISSURA RHINICA

Gyri cerebrales

a. G.O.S. (A), b. G.O.S.(B), c. G.O.S. (C), d. G.O.I., e. G. LINGUALIS

The S.LUN. in Fig. 3 belongs to the first type, one in Fig. 4 to the second type, one in Fig. 5 to the third type.

Fig. 1



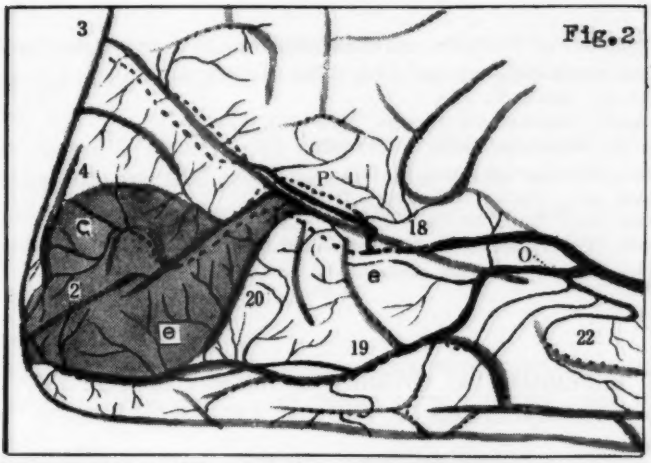


Fig.2
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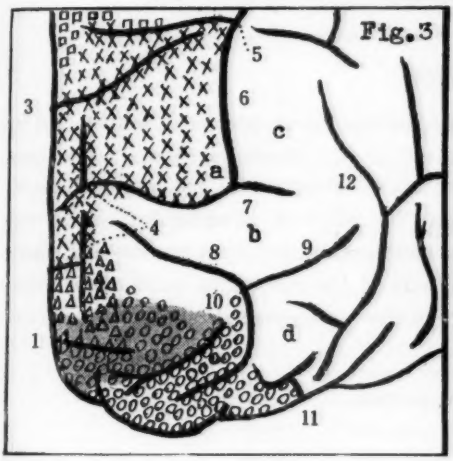
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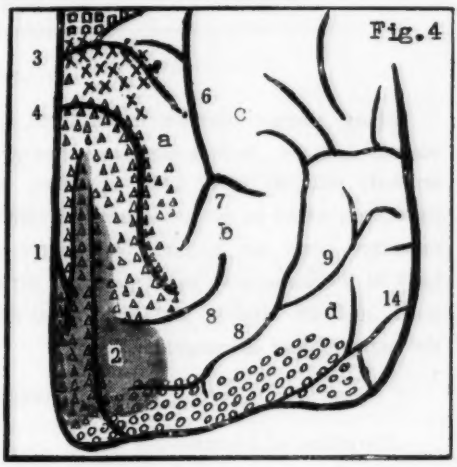
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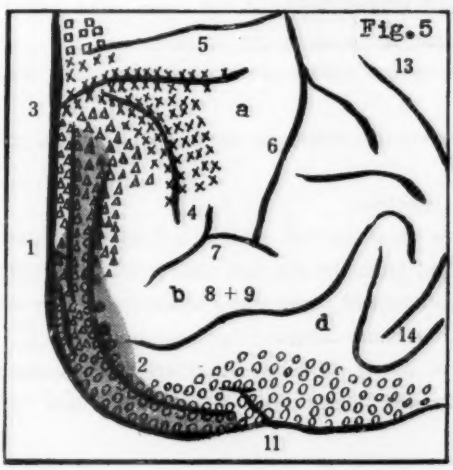
Fig.5
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EXPERIMENTAL STUDIES ON AIIERGY OF THE EYE: PHOSPHATIDES OF THE IRIS AND CILIARY BODY

Shogo KOJIMA

Gifu Prefecluaal Medical School

Introduction

Many allergic diseases encountered in the ophthalmological field have been studied by various authors. It is a matter of course that the allergic disease is caused by antigen-antibody reaction in the body. Proteins, polysacharides, phosphatides and other substances have been found to act as antigen. I have studied the action of phosphatides, which was extracted from the eyeball, as antigen. Phosphatides extracted from the iris and ciliary body of the oxen were injected in the auricular vein of the rabbits to make them sensitized, and the clinical and histological findings in the rabbits caused by the reinjection of the antigen were investigated.

Experimental Method

Extraction of phosphatides

(1) To the iris and ciliary body of the oxen were added the fivefold amount of alcohol, and placed at room temperature for 10 days.

(2) Alcohol soluble, benzol soluble, aceton insoluble, ether soluble and alcohol soluble fractions were made.

(3) Phosphatides were preserved as an alcohol solution, and alcohol was vaporized when used.

Sensitization and re-injection

(a) (1) In order to sensitize the rabbits, 2 cc/kg of emulsion made of bovine serum and phosphatides extracted from the iris and ciliary body was injected repeatedly into the auricular vein of the rabbits five times every other day. Four weeks later, the sensitized rabbits were re-injected the same antigen. (group 1 and 2)

(2) The mixture of 15 mg of BCG, 1.5 cc/kg of phisiological saline and 15 mg of phosphatides of the iris and ciliary body was injected into the auricular vein of the

rabbits twice every other day. Three weeks later the rabbits of tuberculine positive were re-injected with the mixture of 30 mg of BCG, 1.5 cc/kg of 0.85% saline and phosphatides. (group 5 and 6)

(b) Control group 3, group 4 and group 7 received bovine serum, phosphatides of the iris and ciliary body and BCG respectively for sensitization and re-injection. (Tab. 1)

(c) Body weight, blood pictures, Arthus' phenomenon, tuberculin reaction and clinical signs were observed daily, and histology was examined 10-20 days after the re-injection.

Results

Clinical findings

(a) Shock-like symptoms: Shock-like symptoms such as defection, urination and the increase in respiratory rate occurred in all rabbits several minutes after the re-injection, except group 4.

Table I. Experimental Method

rabbit	preparation	provocations
group 1 No. 11. 12. 13.	S+Ps 10 mg 5 times every other day	S+Ps 10 mg
group 2 No. 17. 18. 19.	S+Ps 10 mg 5 times every other day	S+Ps 50 mg
group 3 No. 14. No. 15.	S 5 times every other day	S
group 4 No. 20. 21.	NaCl+Ps 10 mg 5 times every other day	NaCl+Ps 50 mg
group 5 No. 22. 23. 25.	BCG 10 mg+NaCl—Ps 15 mg twice every other day	BCG dead bacillus 20 mg +NaCl+Ps 30 mg
group 6 No. 27. 28. 29.	BCG 10 mg+NaCl+Ps 15 mg twice every other day	BCG dead bacillus 20 mg +NaCl+Ps 60 mg
group 7 No. 24. 25.	BCG 10 mg+NaCl twice eqery other day	BCG dead bacillus 20 mg +NaCl

S:— Oxen's serum 2 cc/kg
Ps:— phosphatides of Oxen's eye
Nacl:— 0.85% NaCl 1.5 cc/kg

Table II. Histological Finding

		rabbits	group 2			group 5			group 6		
			17	18	19	22	23	25	27	28	29
Cornea		vascular dilatation					+		+	+	+
		on the periphery									
		round cell infiltration					+				+
Iris		edematous thickening	++	++	+	++	+	+	++	+	+++
		vascular dilatation	+	+	+	+	+	+	++	++	++
Ciliary body	processes	edematous thickening	+++	++	+	++	+	+	++	+	++
		vascular dilatation	++	++	+	+	+	+	++	++	++
		round cell infiltration	+	+					+	+	++
	corpus	edematous	+			+	+		+	+	+
		round cell infiltration	+			+			+		
Choroid		vascular dilatation				++		+	+	+	+
		round cell infiltration				++			+	++	+

Fig. 1. Ciliary body (No. 17).

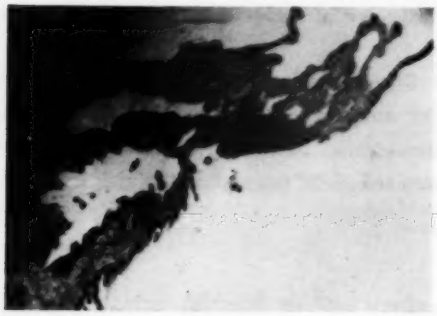

Fig. 2. Ciliary body (No. 29).

(b) Ocular symptoms: In group 1, 3 and 7, hyperaemia of the conjunctiva appeared several minutes after the re-injection which disappeared 24 hours later without any sequel.

In group 2, 5 and 6, hyperaemia and edema of the conjunctiva appeared several minutes after the re-injection which lasted for 24 hours followed with transient alleviation. Five to 7 days later, ciliary injection and redness and edema of the iris appeared again.

In group 4, no signs were observed.

Histological findings

In group 2, 5 and 6, vascular dilatation on the peripheral cornea, edematous thickening and congestion of the iris, coarseness, congestion and round cell infiltration of the root of the ciliary body were observed. Especially in group 5 and 6, conglomeration of the cells in the ciliary body and congestion and round cell infiltration in the choroid were remarkable. (Fig 1,2)

These results are summarized in table II.

No marked hystological changes were observed in group 1,3,4, and 7. The changes in group 2, 5 and 6 indicated iridocyclitis and were significantly different from those in the control groups. Therefore, these iridocyclitis may be considered to be caused by the allergic reaction.

Discussion and Summary

It has been long known that lipids may act as antigen in the allergic reaction. Lipids are actually used in Wassermann's reaction and as Forssman's antigen. Maekawa proved, that the fractions of phosphatides used in Lehman-Facius cancer test had a marked organ specifity on various organs such as the heart muscle, the stomach and the brain. I have extracted phosphatides from the iris and ciliary body by Maekawa's method, and the inflammation of the iris and ciliary body was produced by antigen mixed with antigen proteins, that is, bovine serum and BCG. The inflammation of the iris and ciliary body did not occur in the control groups. By comparing group 1 and 2 with group 5 and 6 the degree of inflammation was found to be parallel to the amounts of phosphatides used. This suggests, that phosphatides are organ specific on the iris and ciliary body. The fact,

that no inflammation nor Arthus' phenomenon was caused by phosphatides alone in group 4 suggests the action of this substance as a hapten.

Bovine serum and BCG similarly produced the mild iridocyclitis. This iridocyclitis may be caused by both serum and bacillus allergy, although there was a slight difference between these histological findings.

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STUDY ON THE SUBRETINAL FLUID
REPORT I. REFRACTOMETRIC AND PAPER-ELECTROPHORETIC STUDY ON THE SUBRETINAL FLUID OF SPONTANEOUS RETINAL DETACHMENT

Atsumi NAKANO

Department of Ophthalmology, Hiroshima University School of Medicine

Introduction

It is considered that the nature of subretinal fluid is depending on the etiology of retinal detachment and it also influences the mechanism of retinal reattachment. The author, therefore, performed the determination of the refractive index and the protein fraction of subretinal fluid obtained after operation, for spontaneous retinal detachment. Attempts were made to pursue comparative study on experimental results, clinical picture and the clinical course after treatment. A similar procedure was also carried out on the vitreous humor in spontaneous retinal detachment and a few cases with other eye diseases for contrast study.

Method

In order to obtain subretinal fluid and vitreous humor, a syringe with a special needle was used. On the needle a ring was made at distances of 1.5 to 3.0 mm. from the tip of it. Subretinal fluid was aspirated by puncturing the subretinal space at the time of operation for retinal detachment. Vitreous humor was collected by puncturing the vitreous cavity at the site of orbiculus ciliaris, when depression of intraocular pressure was required at the time of the scleral resection operation. Abbe's refractometer was used at a temperature of 17.5°C to measure the refractive index. For paper-electrophoresis, Toyo filter paper No. 51 and veronal buffer solution (pH=8.6, μ=0.05) were used. The experiment was arranged as follows: I/cm was read to be 0.24 mA and electrophoresis was

recorded for 10~12 hours. The protein fraction was quantitatively ascertained by using a densitometer which measured the density of fraction recorded on filter paper. This filter paper was prepared by staining with a 0.05% solution of bromphenol blue and was made to be transparent with xylol paraffin. Vitreous humor was concentrated by dehydration according to Mies' method when a large amount of vitreous humor was able to be collected at bulbar enucleation.

Results

Subretinal fluid

Fifteen cases (15 eyes) examined are shown in Table I. In the column of operation, D, S and C represent tear-closing diathermy, scleral resection operation and combined operation of them, respectively. P implies exploratory puncture for subretinal fluid accumulated in cases with severely and totally detached retina. These cases were considered to have very poor prognosis and were not indicated for operation, but by the puncture, a clinical course of changes was observed. Length of time for retinal detachment was estimated with the period from the onset of subjective partial loss of the visual field, to the date of admission to the hospital.

Table I. Cases of Subretinal Fluid measured

Case	State of detached Retina	Retinal Tear	Cystoid degenerated Area	Duration of Detachment	Operation	Postoperative Course
(1) K.K. 64 ♂	Flat	not found	—	3 Months	C.	Abnormal Course, Reattachment
(2) S.K. 59 ♀	Flat	found	—	21 Days	D.	Reattachment
(3) C.H. 59 ♀	Wrinkled	not found	—	4 Months	P.	Failure
(4) Y.T. 68 ♀	Vesicular	not found	—	7 Months	D.	Reattachment
(5) N.O. 54 ♀	Vesicular	found	—	35 Days	D.S.	Reattachment
(6) M.T. 21 ♂	Flat	found	+	16 Days	D.	Reattachment
(7) T.N. 38 ♂	Flat	found	+	23 Days	D.	Reattachment
(8) S.A. 13 ♂	Flat	found	+	5 Days	D.	Reattachment
(9) I.K. 42 ♂	Flat	not found	+	45 Days	S.	Reattrchment
(10) H.H. 18 ♂	Wrinkled	not found	—	6 Months	S.	Failure
(11) T.H. 38 ♂	Flat	found	—	4 Months	S.	Reattachment
(12) T.D. 47 ♂	Wrinkled	not found	—	14 Months	P.S.	Failure
(13) H.I. 59 ♀	Vesicular	found	+	3 Months	D.S.	Abnormal Course, Reattachment
(14) S.Y. 58 ♀	Vesicular	found	+	15 Days	S.D.	Abnormal Course, Failure
(15) T.M. 67 ♂	Wrinkled	not found	—	18 Months	P.	Failure

Table II. Refractive Index and Protein Fraction of Subretinal Fluid

Case	obtained Subretinal Fluid (cc)	Refractive Index	Protein Fraction											
			Praealb.		Alb.		α_1-Gl.		α_2-Gl.		β-Gl.		γ-Gl.	
			M	C	M	C	M	C	M	C	M	C	M	C
(1)	0.1	1.3537	impossible to measure											
(2)	0.15				100	42.8	84.7	9.1	58.0	7.0	38.9	26.1	17.6	14.9
(3)	1.0	1.3769			100	35.7	77.7	6.7	65.0	10.0	49.5	23.0	18.4	24.6
(4)	0.25	1.3355			100	44.4	55.5	10.5	53.8	8.5	40.7	16.8	17.2	19.8
(5)	0.4	1.3394			100	52.7	73.4	6.2	62.6	6.0	31.3	25.9	8.7	9.1
(6)	0.3	1.3359	impossible to measure											
(7)	0.5	1.3372			100	38.1	79.4	6.4	62.6	8.7	47.4	17.2	17.8	29.6
(8)	0.1	1.3351	123.3	1.4	100	41.9	75.9	11.3	61.2	9.0	33.6	25.6	12.1	10.8
(9)	0.5	1.3363			100	48.4	79.8	7.7	68.8	4.7	47.7	24.7	18.3	14.4
(10)	0.35	1.3479			100	40.3	75.5	9.9	55.5	4.9	40.0	14.6	18.2	30.3
(11)	0.2	1.3389			100	27.5	83.3	12.4	68.2	14.3	47.7	24.5	18.2	21.4
(12)	0.15	1.3560			100	33.1	80.7	7.8	65.9	11.5	48.9	19.4	18.2	28.1
(13)	0.42	1.3423			100	44.7	74.1(M)	12.4 (C)			49.4	21.6	18.5	21.3
(14)	0.3	1.3408	impossible to measure											
(15)	0.42	1.3553			100	45.1	62.9(M)	16.0(C)			38.6	15.6	18.6	23.2
mean value					100	41.2	77.6	8.8	62.2	8.5	42.8	21.3	16.8	20.6

Note: M; Relative Mobility
C; Concentration in %

Fig. I. Subretinal fluid of the 10th case.

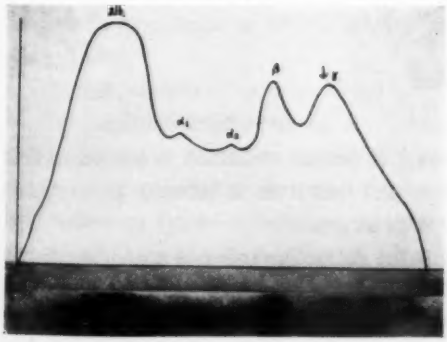
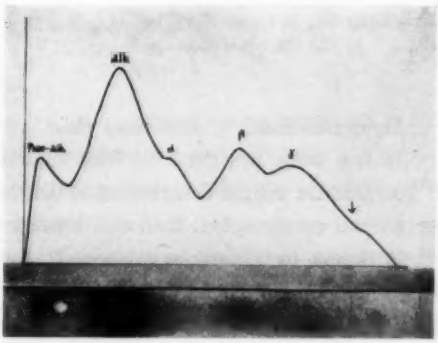


Fig. II. Vitreous humor of the 1st case.



The results obtained for the refractive index and the protein fraction of subretinal fluid are shown in Table II. Figure I indicates the representative features of electrophoresis. Five protein fractions were separated. In the 13th and 15th case, α-globulin fraction was not separated into α_1 and α_2 fractions, and therefore these two cases were not included in the mean value of protein fraction concentration. Measurements of protein fraction in the 1st, 6th and 14th case were not possible due to irregular electrephoretic features.

Vitreous humor

Vitreous humor in six cases (6 eyes) was obtained. In the 1st, 2nd and 3rd case which had spontaneous retinal detachment, subretinal fluid was also sufficiently collected at the same time. These cases correspond to the 12th, 14th and 15th case shown in Table II. The 4th, 5th and 6th case had absolute glaucoma, staphyloma of the cornea and buphthalmos, respectively, and in these cases vitreous humor was able to be collected at operation. The refractive index and the protein fraction of these cases are indicated in Table III. Figure II shows the electrophoretic picture of the 1st case. Five protein fractions were generally separated and the first case was proved to have a prealbumin fraction.

Table III. Refractive Index and Protein Fraction of vitreous Humor

Case	obtained Vitreous Humor (cc)	Refractive Index	Protein Fraction										N.B.
			Praealb.		Alb.		α_1-Gl.		α_2-Gl.		β-Gl.		γ-Gl.		
			M	C	M	C	M	C	M	C	M	C	M	C	
(1)	0.3	1.3363	133.7	9.5	100	39.2			70.5(M)	10.0(C)	44.2	17.9	17.9	23.5	Spont. Ret. Detach Case (12) of Table I
(2)	0.1	1.3354	impossible to measure												Spont. Ret. Detach. Case (14) of Table I
(3)	0.2	1.3358			100	43.9			58.3(M)	17.7(C)	43.3	10.6	18.3	22.8	Spont. Ret. Detach. Case (15) of Table I
(4)	2.2	1.3362			100	43.9	77.3	4.8	67.0	8.3	46.6	21.7	18.2	21.4	Absolute Glaucoma
(5)	0.5	1.3367			100	56.0	79.4	10.6	65.2	4.8	50.0	14.7	18.5	13.9	Staphyloma of the cornea
(6)	2.75	1.3354			100	50.3	72.0	10.0	58.5	5.0	37.8	21.8	18.3	12.9	Buphthalmos

Note: M; Relative Mobility
C; Concentration in %

Discussion

Refractive index of subretinal fluid

It has been described by Hinz that percentage of protein contained in subretinal fluid of spontaneous retinal detachment is 0.4g%～2.6g% and that it is in-between those values for normal cerebrospinal fluid and those measured in serum.

It seems to be definite evidence to show, that the refractive index is controlled chiefly by the amount of protein contained. With the fact that the refractive index of subretinal fluid was estimated by the author to be 1.3351 to 1.3769 in the fifteen cases, it can be said

that the protein concentration of the subretinal fluid shows a great variation in individual cases. A comparative study was also made for clinical features and the refractive index as follow :

(a) The longer the duration of the retinal detachment, the higher was the rate in the refractive index likely to be estimated.

(b) The refractive index tended to be higher in the subretinal fluid of wrinkled detachment than that of flat or vesicular detachment.

(c) In cases with no ophthalmoscopic finding of definite retinal tear, and in those with an abnormal reattaching process or with unsatisfactory improvement after operation, the refractive index was more frequently elevated, than that in other cases.

Refractive index of vitreous humor

The refractive index of the vitreous humor in three cases with spontaneous retinal detachment was approximately equal to that in three other cases. It was slightly higher than the normal value which has been reported by other investigators. However, the refractive index of subretinal fluid collected from the same cases of retinal detachment is far less, and hence, it is noted a great difference in the protein concentration of both fluids.

Protein fraction of subretinal fluid of spontaneous retinal detachment

Hinz, Dorello and Hara have already published reports on the determination of protein fraction of subretinal fluid by means of paper-electrophoresis. It was described by Hinz, that five fractions, namely, albumin, α_1-,α_2-,β-, and γ-globulin were separated in all the eleven cases. To the author's knowledge, however, it is sometimes difficult to separate protein fraction in the subretinal fluid, though it is easy in serum. The author supposes this difficulty could be caused by action of hyaluronic acid in the subretinal fluid.

When the value of the protein fraction concentration of the subretinal fluid is compared with that of serum, the former is far less in albumin and greater in β-globulin, which value is almost the same as γ-globulin in serum.

All the cases were divided into two groups; those well-reattached cases, and poorly or non-reattached cases, even after operation. The mean value of protein fraction concentration was determined in both groups respectively. There is an evidence to suggest, that the latter group tends to have a higher mean value of γ-globulin than that with good-reattachment.

Protein fraction of vitreous humor

On paper-electrophoresis of six cases, four to five fractions were separated in five cases. This was similar to the result reported by Stemmermann and Takaku.

An obvious difference in protein fraction concentration was hardly seen in two cases of spontaneous retinal detachment and the other three cases.

Paper-electrophoretic tracing of subretinal fluid and vitreous humor obtained from the same eye

Only three cases were examined. Considerable similarity of the relative mobility and

concentration of the protein fraction in both fluids, was observed, in spite of great difference in the protein concentration of the two. Therefore, it could be supposed that subretinal fluid and vitreous humor have similar features of protein fractions.

Summary

Attempts were made to measure the refractive index using Abbe's refractometer and to determine the protein fraction by paper-electrophoresis, on subretinal fluid (15 cases) and vitreous humor (3 cases) obtained at operation for spontaneous retinal detachment and vitreous humor (3 cases) collected from other ocular diseases. A comparative study was made on the clinical picture and the experimental results were described as follows:

1. On the electrophoretic tracing of subretinal fluid of spontaneous retinal detachment, five fractions, namely, albumin, α_1-,α_2-,β-, and γ-globulin, were separated in almost all cases.

2. The mean value of the five protein fraction concentrations of the subretinal fluid is 41.2%, 8.8%, 8.5%, 21.3% and 20.6%, respectively, in the order described before. The mean value for albumin is lower, but for β-globulin is higher, than that determined in serum.

3. The refractive index of subretinal fluid ranged between 1.3351 and 1.3769.

4. The refractive index and γ-globulin of the subretinal fluid was found to be greater in cases with a long duration of detachment or abnormal reattaching process after operation, than those in other cases.

5. In the vitreous humor, four to five protein fractions were separated, as done in the case of subretinal fluid.

6. The refractive index of vitreous humor of spontaneous retinal detachment was 1.3354～1.3363, which was slightly higher than that of normal value. It was, however, far less than that of the subretinal fluid of these cases, aud it was almost the same with the value of 1.3354～1.3367, which was seen in the vitreous humor of the blind due to glaucoma.

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FURTHER STUDIES ON SYMPATHETIC OPHTHALMIA WITH ELECTRON MICROSCOPE

Hiroshi IKUI, Kazuo KIMURA and Takeshi NISHIO
Department of Ophthalmology, Kurume University School of Medicine

After our previous report published in 1957[3], we obtained two exciting eye balls with typical sympathetic ophthalmia and observed the ultra-thin sections of the uveal tissues of those eyes under the electron microscope. The purpose of this report is to summarize the whole results of our electron microscopic study of sympathetic ophthalmia in 3 cases, including one mentioned in the previous report. As stated previously, we held on the basis of our observation of the same disease under a light microscope that "sympathetic ophthalmia was a disease of the chromatophores", that the epithelioid cells in this disease were nothing but metamorphosed chromatophores and that "chromato-tropic virus" which had an affinity to the chromatophores might be held responsible for such metamorphosis.[1)2)] Our electron microscopic study of sympathetic ophthalmia has been attempted (1) to clarify the processes of production of melanin pigment in the epithelioid cells and to prove that the epithelioid cells in this disease are nothing but metamorphosed chromatophores, and (2) to find virus-like particles in the epithelioid cells as a causative agent in such metamorphosis of chromatophores.

Materials and Methods

The uveal tissues, especially the choroidal tissue from the 3 enucleated eye balls were fixed in 1 per cent buffered osmium tetroxide, routinely dehydraded in graded alcohol at room temperature, and embedded in a 8 : 2 or 7 : 3 mixture of n-butylmetacrylate and methylmetacrylate. Thin sections for use in electron microscopy were prepared with a glass knife on a Porter-Blum ultramicrotome and examined under electron microscopes (JCM-type 3 : Japan Electron Optics Company ; HS-type 6 : Hitachi).

A part of the choroidal tissue was ground in a glass mortar to be held in suspension in Hanks solution. The suspension was centrifuged and the supernatant was implanted onto HeLa cells in tissue cultures.

Results

(1) *The processes of production of melanin pigments in the epithelioid cells.*

Before entering in details, we may give here our general view on the pathogenesis of sympathetic ophthalmia[1)].

The essential changes occurring in the ocular tissues in sympathetic ophthalmia consist of the proliferation of the chromatophores and the leucocytic infiltration around the chromatophores. The chromatophores become swollen and lose their processes at an early stage of the disease, often showing mitosis and amitosis occurring in them. Proliferating incessantly, they, in due coures, become completely or incompletely converted into so-called epithelioid cells, many appearing in different intermediate forms in the nodular foci of sympathetic ophthalmia. There are also observable a large number of pigment containing multinucleated giant cells of the Langhans type. These giant cells are considered to be derived from the chromatophores when these divide in their nuclei

but not dividing in their cytoplasm. If so, the epithelioid cells must naturally contain the melanin pigments in their cytoplasm.

In a smear preparation of the uveal tissues in sympathetic ophthalmia, the pigment granules in the epithelioid cells show a wide range of morphological variation. Some of them, probably newly formed ones, are so tiny that they are barely visible under a light microscope and others closely packed together to form a coarse lump of pigment. The characteristic finding is that a few dense pigment granules exsist in a dark, less dense area—an area corresponding to the "pigment formation center", as it will be described later. Some epithelioid cells give a weak positive reaction in the presence of dopa.

Electron micrographs of ultrathin sections of the uveal tissues in sympathetic ophthalmia give a clear picture of the pigment formation occurring in the cytoplasm of each epithelioid cell. Presumably, the formation of melanin pigment in its earliest morphological stage is made recognizable by the presence of aggregated minute powder-like granules of low electron density in the cytoplasm, some of the aggregates occurring within the endoplasmic reticulum and the others outside it. These aggregates increase in electron density as they increase in size, and a thin membrane is formed in due time around each aggregate. Fine dense granular masses noticeable in each of the aggregates may conceivably be promelanin. Those promelanin granules grow in size and condense to form melanin pigment. The area in which promelanin granules are found aggregated and a few dense pigment granules formed, is probably identical with the area described by Weissensels (1956)[4] as the "pigment formation center in a melanoblast".

The small melanin pigment granules formed in the pigment formation center increase in size gradually to reach their mature forms, each measuring 0.4 to 0.6μ in diameter and surrounded by a thin membrane; some of these mature forms are homogeneous within and some consisting of droplets varied in size and density and looking granular. These findings put together may be taken to make up the normal process of pigment formation in the chromatophores.

As above mentioned, the epithelioid cells are undoubtedly metamorphosed chromatophores. In an earlier stage of their metamorphosis — the phase of hypertrophy — the chromatophores enlarge and proliferate. However, in the most advanced stage of their metamorphosis — the phase of disturbunce — they undergo degeneration to disintegrate at last. In the degenerating epithelioid cells are presented various pictures showing that the pigment formation is probably disturbed. In the cytoplasm of such a cell are there observable a number of ellipsoid bodies, some containing an almost homogeneous or a powder-like substance of low electron density, and others containing a fine granular material of relatively high electron density with small pigment granules occurring in it. These ellipsoid bodies are conceivable pigment formation centers in a phase of disturbance. The disintegrating pigment formation centers are also often observable. The cytoplasm of a degenerating epithelioid cell contains likewise a lot of degenerating mitochondriae and vacuoles developped from the endoplasmic reticulum.

(*2*) *Pigment granules phagocytized by leucocytes and Hela cells.*

In the foci of sympathetic ophthalmia are occasionally seen some neutrophil leucocytes

or monocytes phagocytizing the melanin pigments released by the disintegration of the chromatophores and the epithelioid cells. The pigment granules found phagocytized in a leucocyte, quite unlike those in an epithelioid cell, are utterly irregular in shape and arrangement, and varied in size, very often disintgrating. Such a peculiar structure as a pigment formation center is, of course, not observable in any leucocyte. HeLa cells in a tissue culture on which the supernatant of the uveal tissue suspension was implanted contain melanin pigment granules in their cytoplasm. These pigment granules in a HeLa cell are also not identifiable with those in an epithelioid cell. These findings show clearly that the pigment granules in the epithelioid cells are not identical with those phagocytized and redistributed in the cells as has been believed by many authors.

(3) *Inclusion bodies in the cytoplasm of the epithelioid cells.*

Some of the epithelieid cells are occasionally found to contain in their cytoplasm one to three basophilic globular inclusion bodies of various sizes, each surrounded by a clear zone. As seen in a smear preparation of the uveal tissue stained with Giemsa solution, the inclusion bodies, measuring 0.5 to 5μ in diameter, are stained blue violet, showing that they are strongly basophilic. Although we stated previously that all inclusion bodies are non-acidophilic, it has now been found that there are some inclusion bodies that stain reddish purple, showing that they are slightly acidophilic though basophilic in nature. Some of the inclusion bodies are homogeneous in structure with a clear margin, and the others seem to contain a few pigment granules.

These inclusion bodies, when seen in electron micrographs of ultrathin sections of the uveal tissues, are found to be composed of a homogeneous substance of high electron density, each containing altered mitochondriae and small pigment granules around or in the matrix, and lined by a membrane that is not clearly visible in some parts.

(4) *Virus-like particles.*

There are groups of some particles around or sometimes in some of the inclusion bodies and also in their neighbouring cytoplasm close to the bodies. The particles are characteristic in their internal structure; they are each possessed of a core (40 to 75 mμ across) of low electron density and enclosed by an opaque shell which is high in electron density and about 25 mμ in thickness; and they range from 65 to 100 mμ in their over-all diameter. In some of the particles the shell has a close fitting envelope around it. There are, besides, disk-shaped particles, each containing a central nucleoid body and being enclosed in a double limiting membrane.

These particles found occurring in the cytoplasm of the epithelioid cells can not readily and finally be identified with virus particles. They are, however, associated with some matrix substance and may be considered to have some relations to the mitochondria apparently altered in nature. They appear in groups, are little varied in size, have a definite internal structure, and are excessively higher in electron density than any other cytoplasmic particles. They bear, furthermore, a close morphological resemblance to the bodies that are believed to be virus particles of influenza by Morgan et al. (1956)[6], and

also to the bodies that are considered by Friedlaender et al. (1956)[9] as virus particles of Ehrlich ascites tumor. The particles with a central body resemble the mature form of virus particles of vaccinia described by Morgan et al. (1954)[5]. These evidences suggest the possibility that these are virus particles.

The characteristic finding is that there is a matrix substance closely resembling the "matrix region" described by Gaylord and Melnick (1953)[7]. They noticed the presence of a matrix substance of moderate electron density in the ectodermal cells of the chorio-allantoic membrane of a developping egg infected with Ectromelia virus, and found that the substance contained virus particles in the various stages of their development. The matrix region found in each epithelioid cell is also made up of an ill defined mass of a matrix substance of moderate electron density. This region contains virus-like particles, 60 to 80 mμ in diameter, which do not show any distinctive internal structure. Whether these particles are unmature forms of virus or not remains unsettled. What is interesting in this connection is that Wilczek and Feltynowski (1955)[8] found under an electron microscope some virus-like particles in the ultracentrifuged form of the intraocular tissues with sympathetic ophthalmia. The specimens in which they observed the particles were prepared by Cox's method. The virus-like particles they discovered in these specimens were small oval bodies and large composite particles, measuring 60 mμ and 100 to 160 mμ in diameter, respectively. More research is needed, of course, before the final identification can be made.

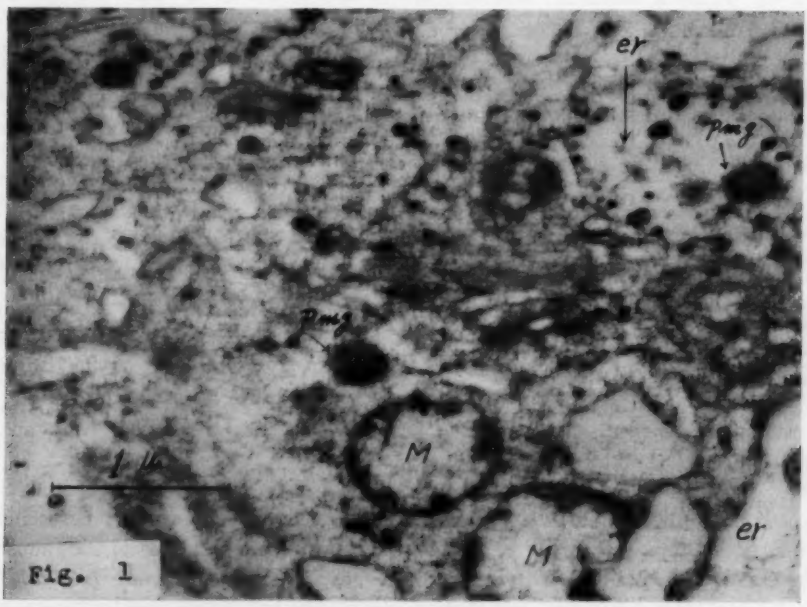

Fig. 1. Aggregates of promelanin granules in the cytoplasm of the epithelioid cell (metamorphosed chromatophore). M: mitochondria, er: endoplasmic reticulum, pmg: promelanin granules. ×30,000.

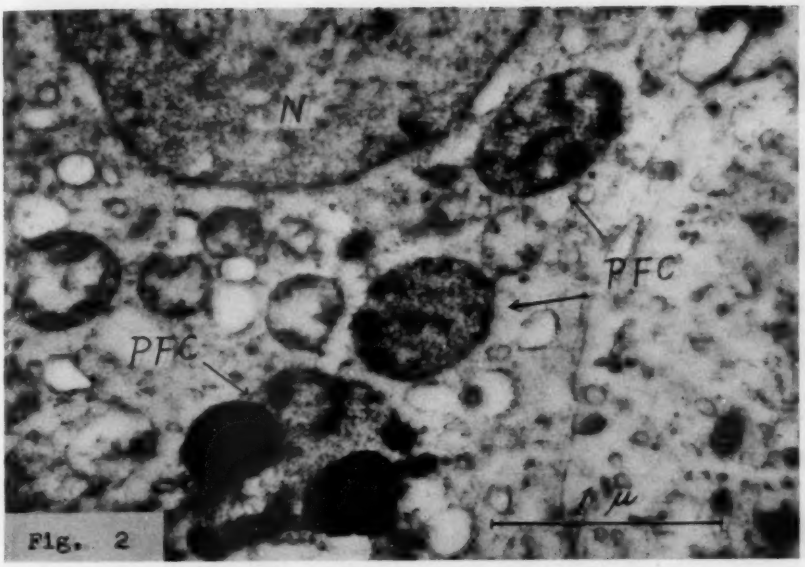

Fig. 2. Pigment formation centers and melanin pigment within them. Melanin pigment granules appear to be formed by condensation of promelanin granules.
N: nucleus, PFC: pigment formation center. ×40,000.

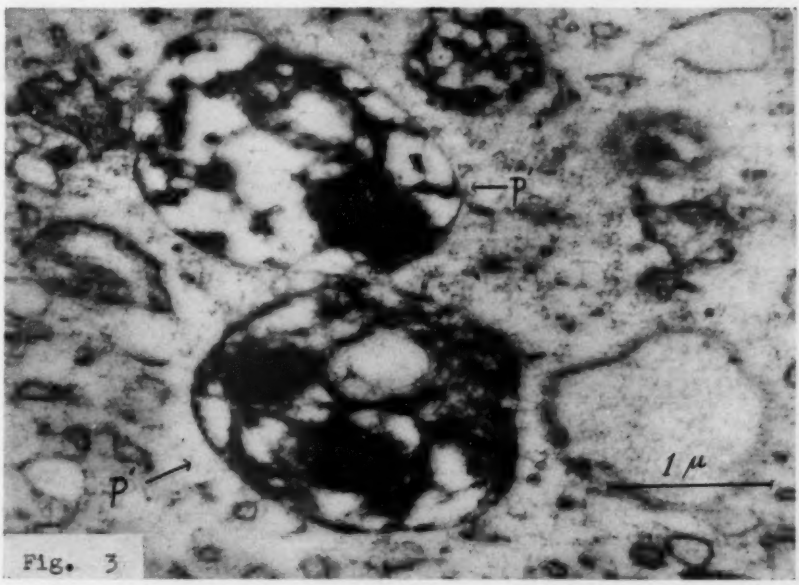

Fig. 3. Disintegration of pigment formation centers. In the later stage of the metamorphosis of chromatophores, the pigment formation centers disintegrate.
P': disintegrating pigment formation center. ×30,000.

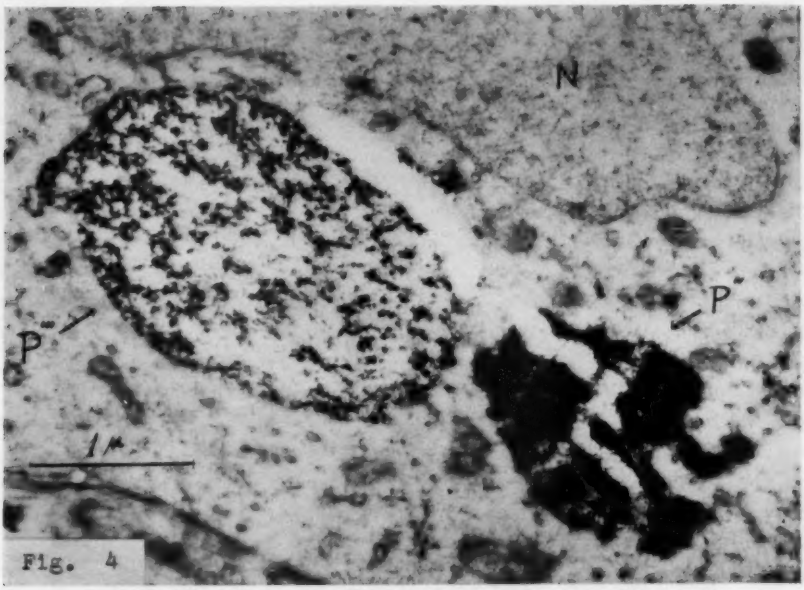

Fig. 4. Disintegration of pigment formation centers. P''': disintegrating pigment formation center, P''': disintegrating hypoplastic pigment formation center. × 30,000

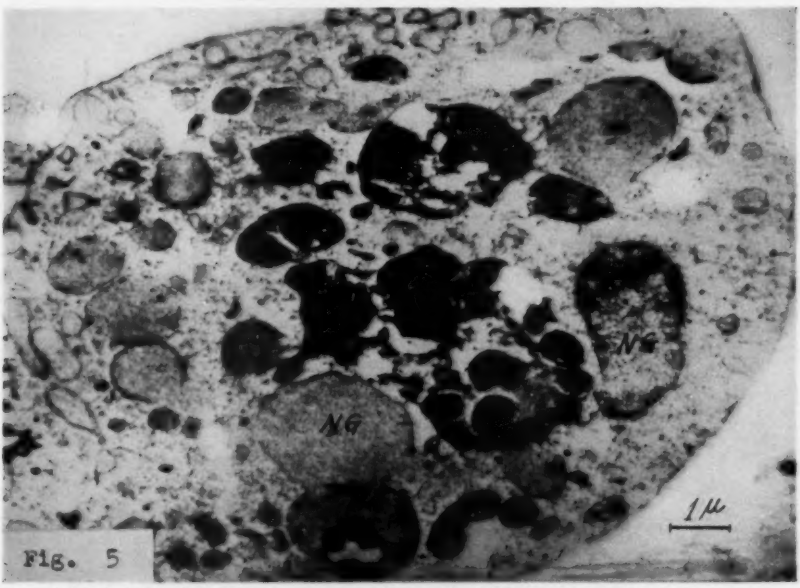

Fig. 5. Melanin pigment granules phagocytized in the neutrophil leucocyte. The pigment granules phagocytized in the cytoplasm of the neutrophil leucocyte are disintegrating. NG: neutrophil granules. ×11,000.

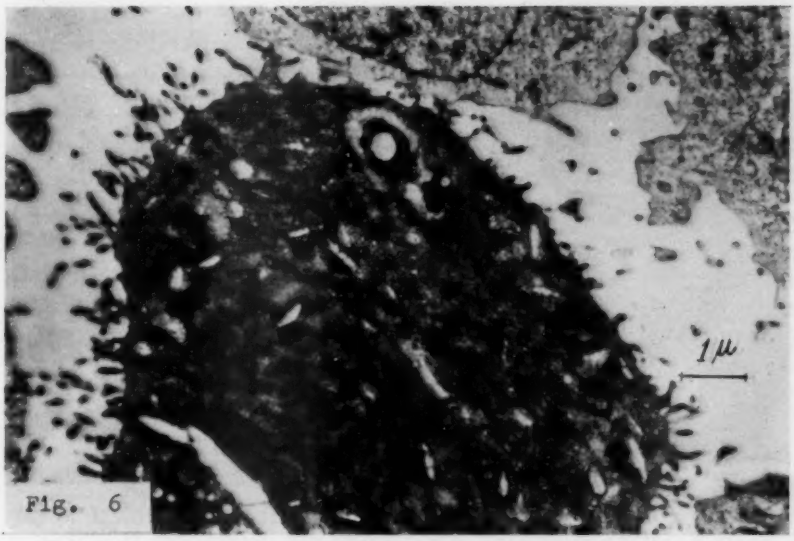

Fig. 6. Melanin pigment granules taken in the cytoplasm of HeLa cell. ×11,000.

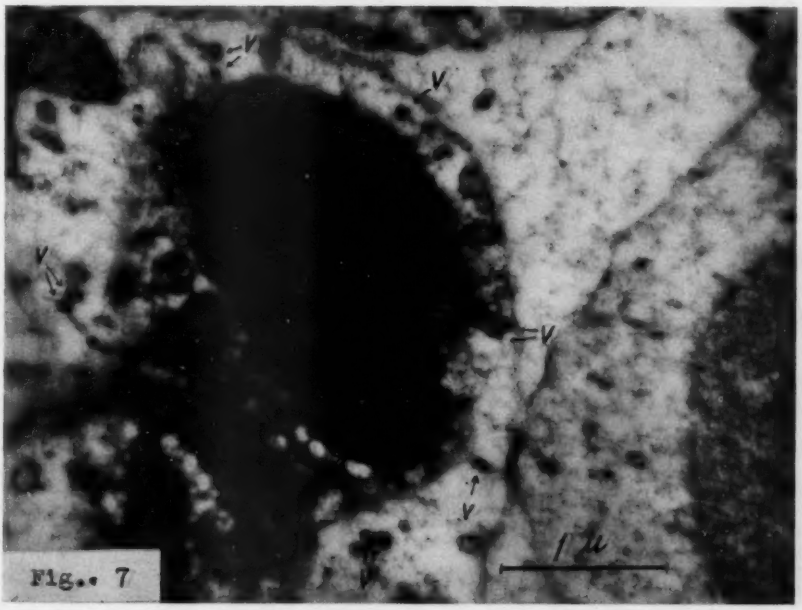

Fig. 7. Cytoplasmic inclusion body in the epithelioid cell (metamorphosed chromatophore). Virus-like particles are observable around the inclusion body.
V: virus-like particles, M: mitochondria. ×30,000.

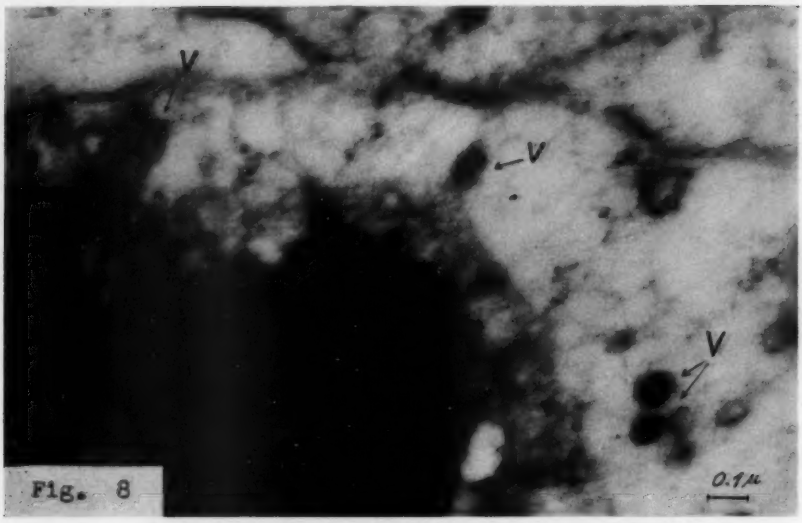

Fig. 8. Part of the preceding figure at higher magnification. The particles at the left and the middle are composed of a central body (nucleoid) and the double limiting membranes. ×70,000.

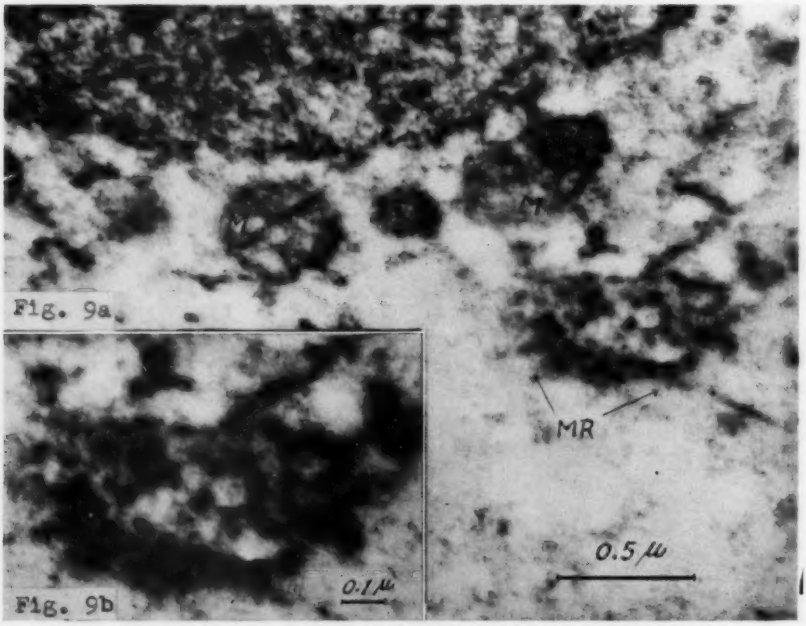

Fig. 9a. Matriix region and the virus-like particles. M: mitochondria, V: virus-like particles within the matrix region. ×45,000.
Fig. 9b: Higher magnification of the matrix region. ×82,000.

Summary

1. Electron microscopic studies in ultrathin sections of the uveal tissues in sympathetic ophthalmia were performed in 3 cases. The cytoplasm of some epithelioid cells presented various pictures of the formation of melanin pigment occuring in it. The pigment granules in these cells are quite different from those phagocytized by leucocytes and by HeLa cells. These facts, together with the light microscopic findings obtained in the same disease, prove that the epithelioid cells in sympathetic ophthalmia are nothing but metamorphosed chromatophores.

2. The indubitable and invariable presence of virus-like particles in the matrix region as well as in and around the cytoplasmic inclusion bodies in some epithelioid cells—a fact observed in the eye balls with sympathetic ophthalmia—has enhanced the possibility that the causal agent of this eye disease may be a chromato-tropic virus.

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EIECTRON MICROSCOPY OF THE RETINAL VESSELS
REPORT I. HUMAN RETINA

Jobu MAEDA

Department of Ophthalmology, Kurume University School of Medicine

Introduction

On its embryological aspect the retina consitutes a part of the central nervous system, so that its blood vessels and capillaries, like those of the brain and spinal cord, exist within nervous tissue and are supported by glia. It has, therefore, been assumed that the retinal vessels bear a close resemblance to the cerebral vessels and differ in structure from those of any other part of the body.

It must be admitted, however, the histological knowledge so far gained about the retinal vessels, about the retinal capillaries, in particular, is far from sufficient, the only available in formation on this subject being the description by Krückmann, Evans, Walter, Uyama et al. of the morphological relation the retinal vessels bear to the glia and to the surrounding nervous tissue and the function of the walls of those vessels, which they explored under the light microscope.

The structure of the walls of these vessels and the delicate internal structure of the

component cells of those vascular walls are practically left unexplored in many respects.

The present work was undertaken with a view to clarifying under the electron microscope the structure of the retinal vessels and capillaries and the relation these bear to the surrounding tissue.

Material and Method

Material. Normal retina from a 53-year-old male showing, clinically, no pathological change in his eye balls, the retina and all else.

Method. The material was treated by Caulfield's method: it was left immersed for 30 minutes in 1% osmium veronal buffer solution for fixation; washed with water and dehydrated; and embedded with n-buthyl-methacrylate containing 1-1.5% of its volume of benzoyl-peroxide.

Observation

The electron microscopic picture of the retinal vessels makes it necessary to describe the blood capillaries and the fine blood vessels separately.

1) Capilllaries

Under an electron microscope, as under a light microscope, the retinal capillaries are seen distributed in three tissue layers: The nerve fibers layer, The ganglionic cell layer, and inner and outer plexiform layer. The capillary walls, each consisting of endothelial cells, a basement membrane forming the outer layer of the endothelial cells and of pericytes and lined with endothelial cells, are surrounded by a glia with nervous tissue occurring on its outer side (Fig. 1,2, and 3).

Endothelial cells

The endothelial cells are flat-bodied and arranged in a single layer; in its cross section the cell body is greatly varied in thickness in its different parts (ranging from 0.1μ to 3μ).

Those forming the lumen of the vessel have a small number of short microvilli on their free surface and form a terminal bar between each two of them to be closely connected one to its neighbors. This terminal bar is a collection of electron dense particles (Fig. 4a, 4b).

Seen in a specimen tissue of the retina, the body of each endothelial cell is continuous (from and to end) and not marked with a pore in any part of it, though it is thinned down here and there; it is kept in close contact with the basement membrane, no space being left between them; it has a large number of roundish and nearly equal-sized vesicles occurring in its cytoplasm—pynocytic vesicles noted for the first time by Palade, and found rather regularly arranged in the close inside proximity of the outer and inner cell membranes or occasionally attached to the membranes to form with them an alveolar structure reffered to as caveola intracellularis (Fig. 5.)

Basement membrane and Pericytes

The basement membrane is in close contact with endothelial cells on its inner surface and with the nearest glia on its outer surface and has a large number of pericytes within it, so that it is split in many parts to present a complicated appearance as a whole, and

greatly varied in thickness in its different parts (ranging from 500Å to 1μ) (Fig. 7).

Filled with a substance homogeneous in consistency and medium in electron density, the membrane is low in density (250Å-300Å) in its thin layer adjacent to the neighboring cells on either side, and higher in density in its inner part, where it is either a defined and delicately fibroid or laminated in structure. It is, besides, structureless in parts, a vacular empty space varied in size occurring here and there within it. A sight which may or may not be ascribable to an artefact produce during the process of fixation or of embedding (Fig. 8).

The pericyte is highly irregular-shaped on its cut surface, probably because it has dendriform or stellate processes. A large number of pericytes, each hidden altogether under a basement membrane and superimposed, like a tree branch. on its neighbor, are formed into a layer and, together with the bassment membrane, surround the endothelial cells. These pericytes have pynocytic vesicles in their cytoplasm (Fig. 6,7).

Glia

The glia cell has a body branched in various ways and each capillary has around it several glia cells, each with a large body and with a terminal limb extended far into the surrounding nerve tissue. It occasionally happens, that a capillary appears enwrapped half along its length by a single glia cell. A glia cell surrounding a capillary is conspicuously varied in thickness in different parts of the capillary and has a globular or semiglobular nucleus in it, often near here the cell is in contact with the capillary (Fig. 1,2, and 3).

The glia cell is much lower in electron density in its cytoplasm than many other cells and the cytoplasm contains equal-sized vesicles and highly electron dense minute particles in large numbers. These glia cells are varied in their internal sturucture: the same have a small number of minute particles in them and are very low in electron density in their cytoplasm; some are fibrous and low in density in their cytoplasm (Fig. 4); and others have closely packed minute particles, and are of high density in their cytoplasm. (Fig. 9).

2) Small Blood Vessels

The small vessels are divisible, though with much difficulty, into arterioles and venules from the thickness and structure of their walls and from the diameter of their lumen.

Arterioles.

The basement membrane of an arteriole is much thicker (6μ-10μ) than that of a venule and identical in its microstructure with that of a capillary, with smooth muscle cells scattered here and there within it. This basement membrane is a continuous and non fenestrated layer, with collagen fibrils layered outside it. The smooth muscle cells resemble the pericytes of a capillary in that their cytoplasm is homogeneous. (Fig. 11).

Venules.

The basement membrane for a venule is thin, with pericytes, but not any smooth muscle cell, occurring within it. A venule differs from a capillary in that it is larger in lumen and has a layer of collagen fibrils on the outer side of its basement membrane. This

layer of collagen fibrils is surrounded by glia cells and has a loose-packed stroma low in density, with close-packed fibroblasts in the form of minute particles occurring within it. (Fig. 10).

Discussion

In a retinal capillary the endothelial cells, just like those in a cortical capillary (Maynard et al.), have each a body neither broken in the middle nor fenestrated even in its thinnest portion, as seen in a cross section. Each retinal capillary is different in the arrangement of its basement membrane and pericytes from a capillary in any other tissue of the body: the basement membrane is split in various ways and varied in thickness in its different portions under the pressure of an enormous number of pericytes contained in it.

According to Maynard et al, the basement membrane of each cortcial capillary contains cells with a fragmentary body and such a cell is, according to Maynard et al., again, a probable prototype of the smooth muscle cell. The pericytes of any retinal capillary are homogeneous in their cytoplasm except, where a pynocytic vesicle occurs, and bear a close resemblance in structure to the smooth muscle cell. The two different cells are, indeed, very alike in structure and are both characterized by the fact that they are found in a basement membrane, making it highly probable, as pointed out by Maynard et al, that the former is the prototype of the latter and that the two are identical in function. However, with all its points of resemblance to a cortical capillary, a retinal capillary is distinguishable from a cortical capillary in that it has a thick basement membrane, is varied in thickness in its different portions, and abounds in pericytes.

The small vessels and capillaries supplying that retina are surrounded all over by glia and it is not likely, that their walls are in direct contact with any of the local nerves.

The surrounding glia is in part composed of Müller's fibers extended in part to the wall of the capillary, though Müller's fibers and astrocytes, so alike in the microstructure of their cytoplasm, are practically indistinguishable in the capillary wall.

Arterioles and venules supplying the retina are not distinct from any other similar blood vessels of the body except in that the former are, and the latter are not, surrounded by glia. In this respect a retinal small blood vessel is identical in structure with a cortical one, leaving no interstitial space around it.

Conclusion

The blood vessels and capillaries in a normal human retina were histologically studied and compared under the electron microscope with those in other parts of the body, the cerebral cortex, in particular.

1. A retinal capillary is composed, to mention the lowermost first, of a layer of endothelial cells, a basement membrane and pericytes, with glia occurring around it.

2. Each endothelial cell, with a body neither split nor hollowed in any portion of it, is closely connected to its neighbors by a terminal bar and so closely attached to the basement membrane that there is no interstitial space left between the two.

3. The basement membrane, thicker than the one of any other tissue, is split in various

ways in its layers and has pericytes, stellate or dendriform, enveloped in it.

4. The glia surrounding a retinal vessel and capillary along their whole length is morphologically divisible into two or three kinds.

5. Each arteriole and venule of the retina has a collagen fibril layer on the outer side of its base membrane.

6. The basement membrane of a arteriole contains smooth muscle cells and is not fene- strated.

7. The retinal vessels and capillaries, though closely resembling those of the cerebral cortex in structure, are a little different from the latter in the arrangement of the glia and in the form of the basement membrane and of the pericytes.

(Published in Acta Ophth. Jap. 62:1002, 1958 in Japanese.)

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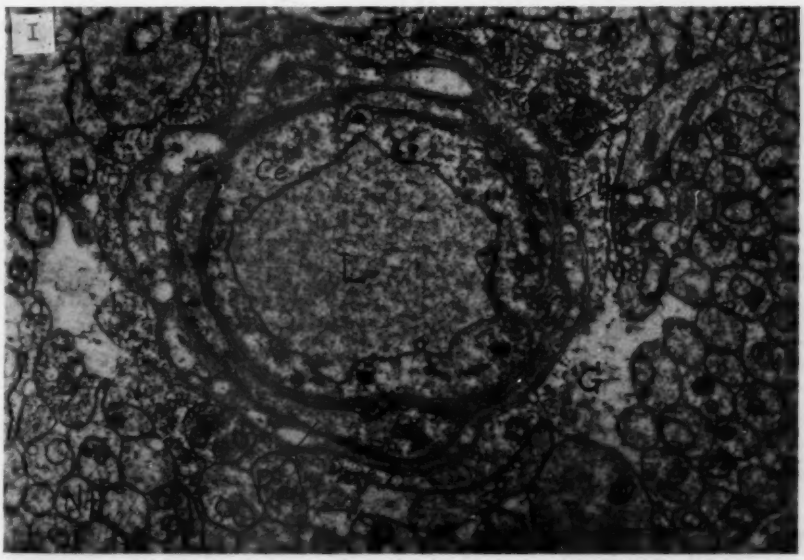

Fig. 1. A retinal capillary in the nerve fiber layer. The capillary is composed of a single layer of endothelial cells (Ce), a basement membrane (Bm) and pericytes (P). G:glia. Nf: nerve fiber. × 18,600

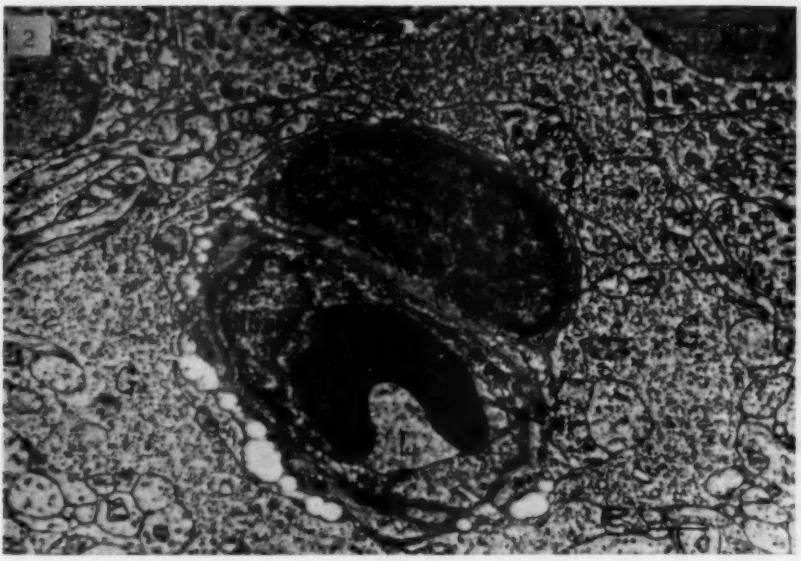

Fig. 2. A retinal capillary in the outer plexiform layer of the retina. G: glia cells are large-sized, each extended over half the circumference of the capillary, and are varied in electron density; some of the red blood cells (R) seen in the lumen are in close contact with the coating membrane of an endothelial cell. mv: microvilli. × 9,600

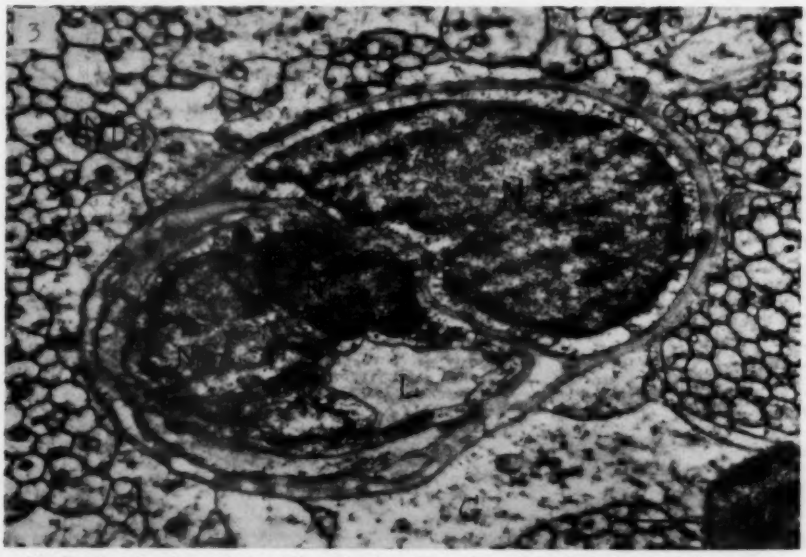

Fig. 3. A retinal capillary. The cytoplasm is narrowed in diameter and the lumen (L) in the part where the nucleus lies. NG: nucleus of a glia cell. × 12,600

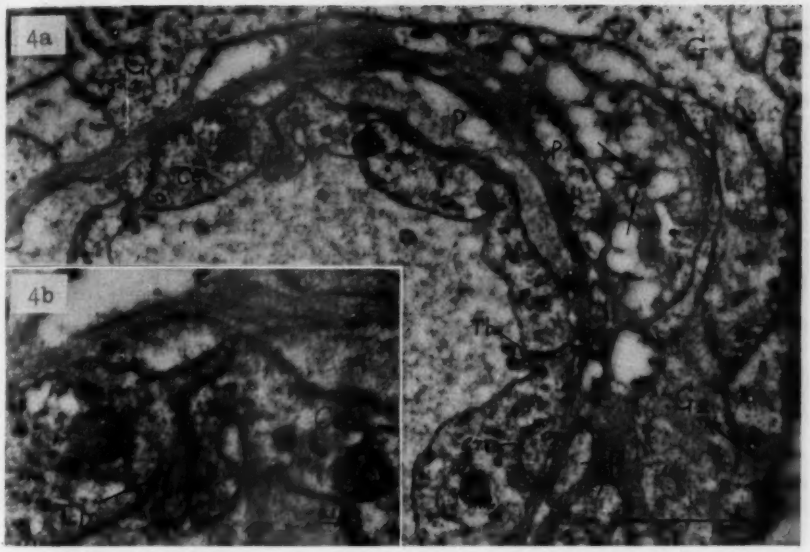

Fig. 4a. A retinal capillary. Each endothelial cell, is kept in close contact with neighbors to form a terminal bar (Tb); the body of the cell is, as seen in a cross section, is continuous from end to end, with no pore in it, there is no spase left between the cell and basement membrane. Arrow: A glia cell (G2) higher in electron density than the others. and fibrous in its microstructure. V: A vacuole in the basement membrane. × 24,600

Fig. 4b. A terminal bar magnified. This is an irregular-shaped elevation in the lumen of a vessel. ×49,200

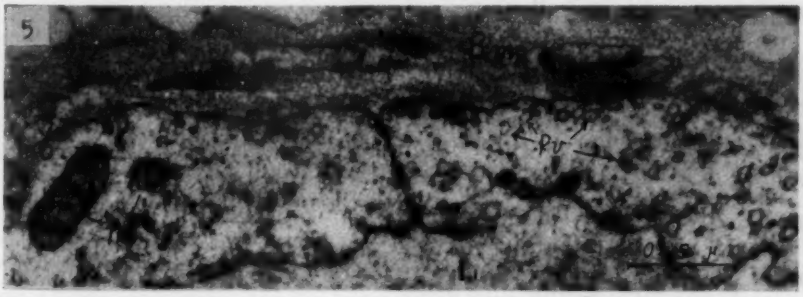

Fig. 5. Pynocytic vesicles (pv) in the cytoplasm of an endothelial cell. These are seen in large numbers, some of them attached to the cell wall and alveolar in structure. ×37,800

Fig. 6. Pynocytic vesicles occurring in the cytoplasm of a pericyte. × 37,800

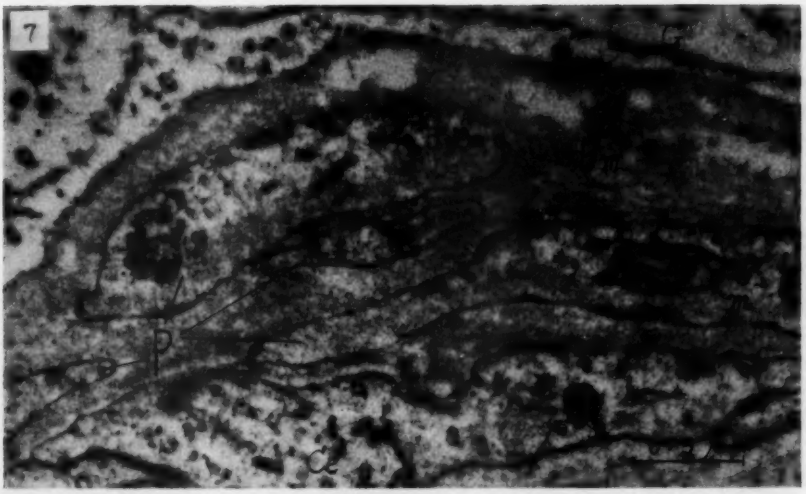

Fig. 7. Pericytes entirely enveloped in basement membrane. The cell bodies, superimposed one upon the other, are split in different ways and greatly varied in thickness in their different parts. × 37,800

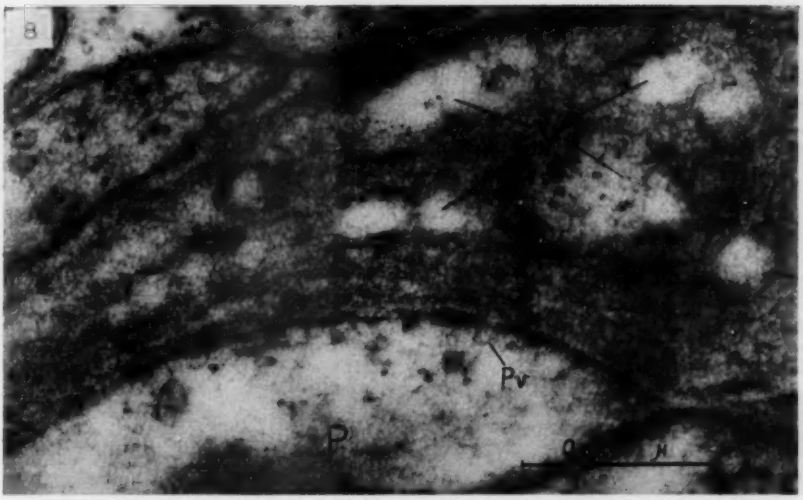

Fig. 8. Basement membrane filled with homogeneous substance. It is low in density where it adjoine neighboring cells, and high in density in the central part where the it is fibrous and laminated in structure. v: vacuoles in the basement membrane. × 64,800

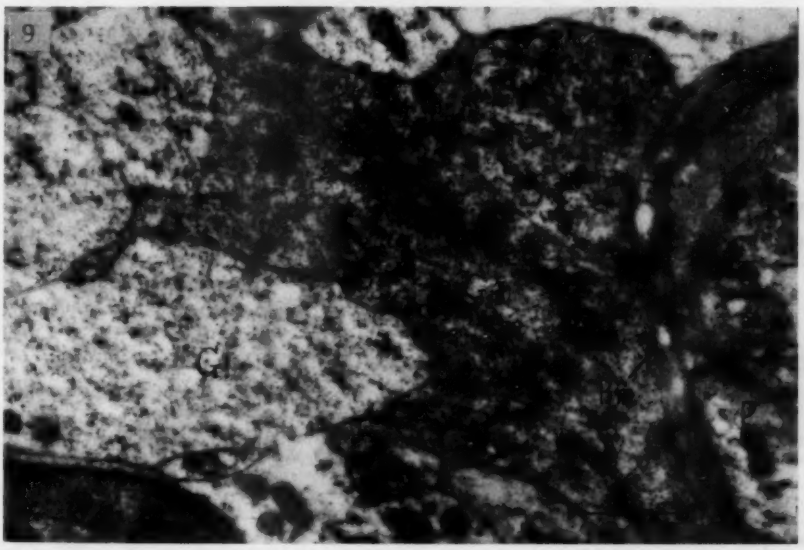

Fig. 9. Glia cells. These are high in density in their cytoplasm in which minute particles are seen closely packed together. These resembled microglia cells found in the cerebral cortex. × 19,200

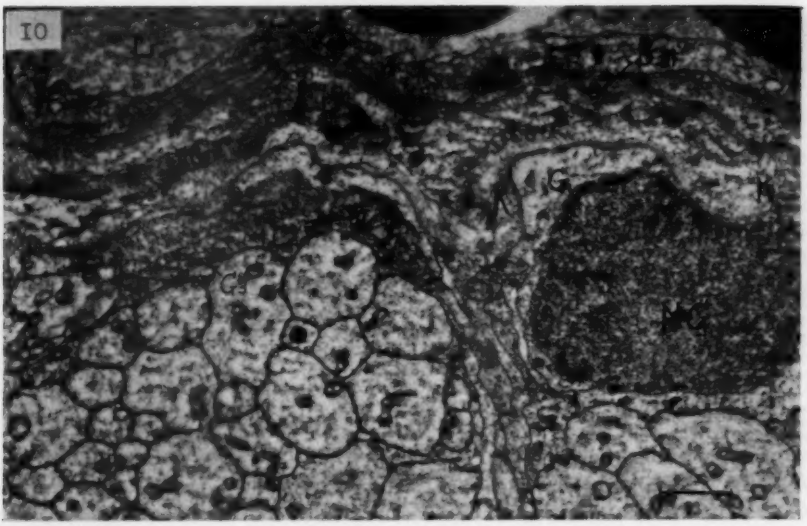

Fig. 10. Venules. The basement membrane is thin, measuring from 0.15 μ to 3μ thick, each with a collagen fiber layer (K) all around it. F: fibroblasts within a collagen fiber layer. G: glia. × 12,600

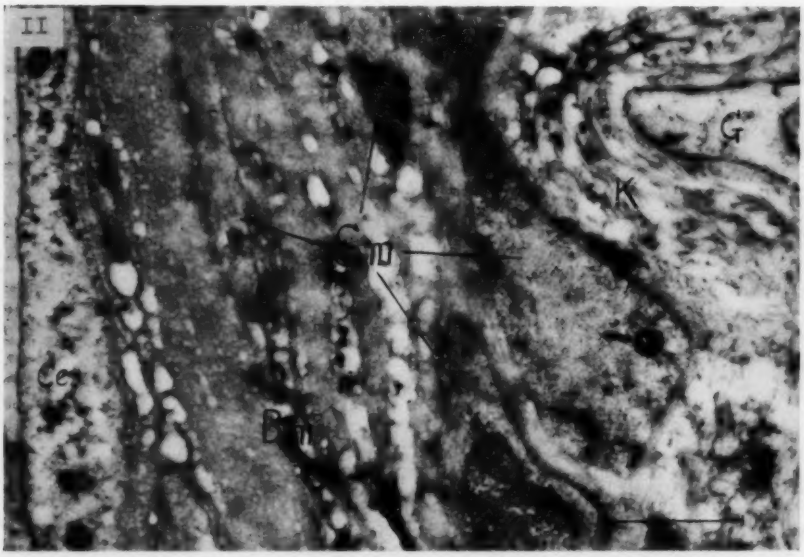

Fig. 11. Arterioles. The basement membrane is very thick—6 μ to 10 μ thick, with smooth muscle cells (Sm) arranged discontinuously within it. K: collagen fiber layer. G: glia. × 25,200

ELECTRON MICROSCOPIC STUDY ON THE FINE STRUCTURE OF RETINAL PIGMENT EPITHELIUM AND CHORIOCAPILLARY LAYER

Shinobu IWAKI

Department of Ophthalmology, Kurume University School of Medicine

The structure of pigment epithelium, together with its functional relationship with visual cells, was studied intensely by light microscopists[1-3].

There were also several reports of electron microscpic studies of the structure[4-7]. They were concerned, however, chiefly with parts of the structure, e.g. isolated fuscin granules, but not with its overall feature. It seemed to the author therefore to be worthwhile to study its fine structure with the electron microscope in relation to studies reported hitherto.

Materials and Methods

The bulbus of the human, mouse, rabbit (albino) and uroloncha striata, removed with appropriate procedures, were fixed with the modified Palade's fixative[8] for 30 to 60 min.. After washing for 30 min., they were dehydrated by serial concentrations of ethanol and were embedded in n-buthyl methacrylate, mostly only or partly with 5% of methyl-methacrylate, containing 1-2% benzoyl peroxide. The plastic was polymerized at 45°C. The microtome used was JUM-3 type of Japan Electron Optics Co. and JEM-4c microscope of the same Co. was used for examinations.

Observations

At the free surface of the epithelium, numerous processes project toward the visual cells. Two kinds of them are distinguished; one is the fine and long one and the other is the big and short one. The former microvilli-like processes surround the lateral sides of the outer segments of visual cells (Figs. 1,3,4,5,6,7 and 8), extending further toward the inner segments and the outer segments many times rest upon the tops of the latter short processes (Figs. 1 and 5). No direct connection is recognized between the processes and visual cells although very close contacts are commonly observed.

The lateral cell surface is bounded smoothly without interdigitation and its distal parts shows the structure of the terminal bar of the epithelium[9] (Fig. 4).

The basal cell border posesses an intense plication, showing the double membrane nature at sections because of infoldings or invaginations of the border membrane (Figs. 1,2 and 4).

The nucleus is ovoid-shaped and occupies the central portion of the cell (Fig. 1). The rod-shaped mitochondria are found usually beneath or at the sides of the nucleus and show clear cristae mitochondriales.

One feature of the cell is full of the endoplasmic reticulum in the cytoplasma. Most of the reticulum is not accompanied by Palade's particles and shows a so called smooth surfaced nature (Figs. 1-7), although at some parts one can recognize the rough surfaced reticulum with Palade's particles (Figs. 3 and 4). Golgi apparatus can also be observed sometimes at the sides of the nucleus but it is somewhat difficult to differentiate the apparatus from the reticulum because of the similar membranous natures of these two structures.

The fuscin granules of high electron density are obvious in man (Fig. 5) and uroloncha striata (Figs. 7 and 8). The careful observation of granules at higher magnifications gives the information that small units of particles, or in addition to them fine networks in the case of uroloncha striata, are embedded in the matrix of the granule. The limiting membrane is sometimes recognizable around the granule but no thread-like attachment is observed in pictures so far obtaind.

In the case of uroloncha striata, a lamellar structure of somewhat crescent form is observable.

This structure appears to be the lamellar pile of paired membranes, each pair of about 40 Å thick membranes with about 40 Å interspace being apart about 120 Å, which seem to be continuous to the surrounding endoplasmic reticulum (Fig. 7).

Sometimes at the roots of microvilli-like prosesses of the epithelium of the rabbit, six or possibly three pairs of small dense granules arrange like petals of a blossom. Their relationship with the surrounding endoplasmic reticulum or the three dimensional structural relationship of these granules to each other are not clear at present.

The endothelial cell of the capillary of the choriocapillary layer is extremely thin and shows abundant fenestrations such as observed in the renal glomerulus[10] chiefly at the side facing to the epithelium (Figs. 1 and 2) and the nucleus of the cell does not usually set its position at this side. Between the epithelium and the capillary is recognized the narrow connective tissue layer with collagenous and elastic fibers.

Discussion

Some light microscopists considered, that the processes of the pigment epithelium directly connect with the cytoplasm of the visual cell but the present electron microscopic data deny this consideration. The big difference of the resolving powers of instruments used will explain this contradiction.

The intense plication at the basal cell border and the abundant fenestrations of the capillary endothelium suggest some active metabolic relationship between the epithelium and the capillary.

The smooth surfaced endoplasmic reticulum is so full, that the cytoplasm appears quite unique being much different from the common cell types. Although its function is not clear at present, the fact suggests that the cytoplasm as a whole must have a very specialized activity.

The inner structure of the fuscin granule is difficutult to be studied because of its mechanical hardness and its high electron density but occasional chances show at least, that it is not homogeneous but embeds small units of particles or fine tubular networks in it. This fact may be useful in thinking of the developmental process of the granule. Many authors considered that a thread or string-like filament attaches itself to the one end of the granule but all of these studies were carried out for isolated granules. Although the three dimensional configuration is not clear, the present study does not affirm the existence of such a filament in any kind of eye so far examined.

The lamellar structures observed in the case of uroloncha striata seem to correspond with the crescent structure which Miyake[11] considered under light microscope as one growing stage of the myeloid body.

Summary

Sections of retinal pigment epithelia and choriocapillary layers in eyes of man, mouse, rabbit (albino) and uroloncha striata fixed with the buffered osmium tetroxide solution, are observed with electron microscope and conclusions are obtained as follows.

1. The common facts in above animals are the presence of a kind of microvilli-like processes on the surface of the cells, the visual cell side in the retinal pigment epithelium and lack of direct shift from the cytoplasmic membrane of microvilli-like processes to that of visual cells, although the processes are wrapping the lateral side of visual cells.
2. The fuscin granules of man have 2 types, however, an intermediate type is also present.
3. The fuscin granules of the uroloncha striata have an indistinct architecture and fine tube-like bodies form a network in the substance which has high electron density.
4. The endothelial cells on the choriocapillary layer are very thin and at this site, many fenestrations or pores are present.

(This article was published in Acta Societatis Opthalmologicae Japonicae Vol. 62, p. 995 (1958) in Japanese language.)

Explanations of Figures

All figures are electron micrographs of pigment epithelia and capillaries of choriocapillary layers, or parts of them.

Mark:

Ce; capillary endothelium
Cl; capillary lumen
Co; cone outer segment
err; rough surfaced endoplasmic reticulum
ers; smooth surfaced endoplasmic reticulum
F; fuscin granule
I; plication at the basal cell border
m; mitochondria
mv; microvilli-like process
N; nucleus
NL; nucleolus
P; fenestration in the capillary endothelium
Ro; rod outer segment

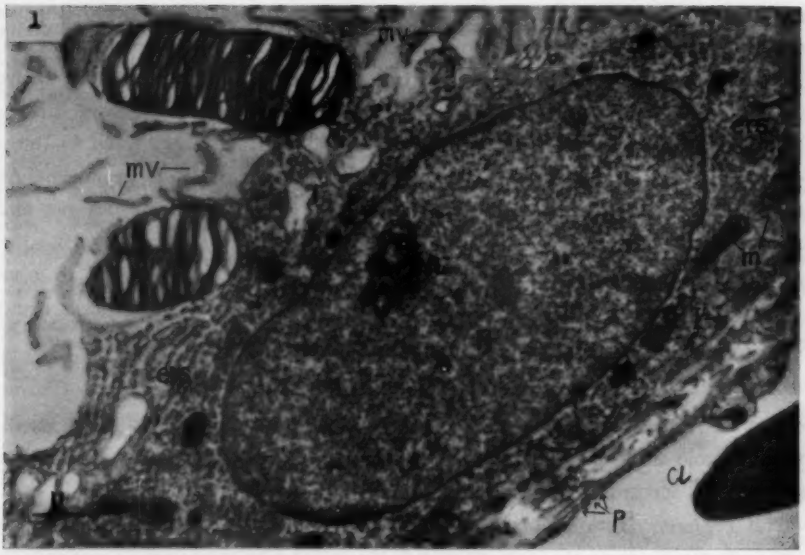

Fig. 1. Mouse. A nucleus is observed in the center of the cell and a number of mitochondria are recognized beneath or at the sides of the nucleus. The microvilli-like processes exist on the cell surface (mv), covering the lateral side of the rod outer segment (Ro) in visual cell. × 9,000.

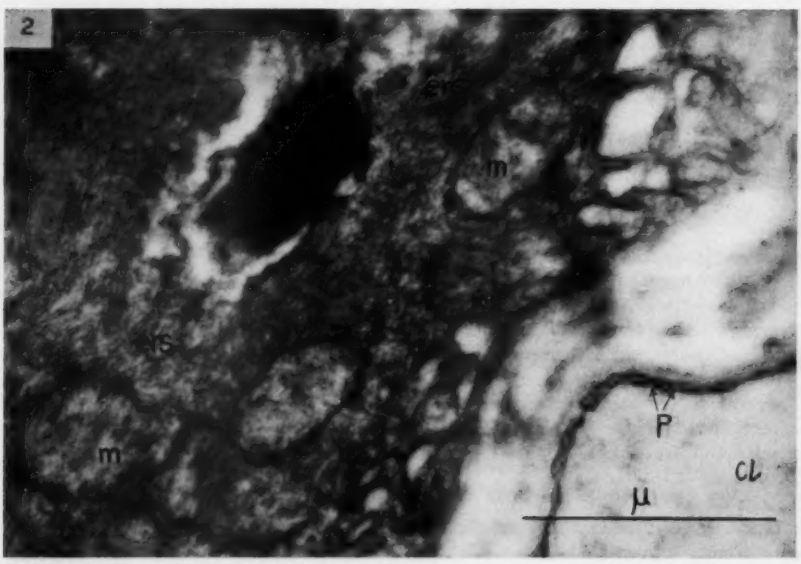

Fig. 2. Rabbit. The cytoplasm is filled with nets of smooth surfaced endoplasmic reticulum (ers) and the plication is observed at the basal cell border. The arrow points the fenestration or pore, of which quite a few are existing in the capillary endothelium. ×44,000.

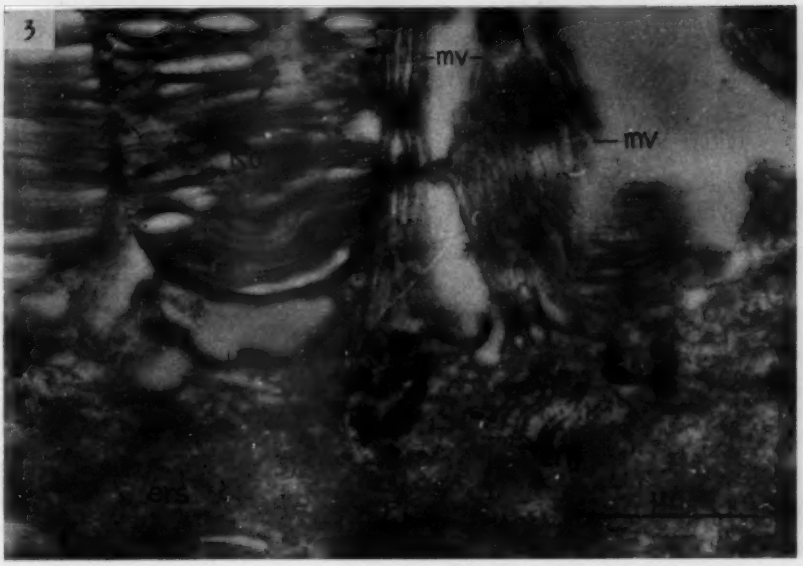

Fig. 3. Rabbit. The correlation of the rod outer segment and the microvilli-like processes, At the root of the microvilli-like processes, six or possibly three pairs of small dense granules are observed in the petals of the blossom like arrangement. In a part of the cytoplasm, rough surfaced endoplasmic reticulum is observed. × 32,000.

Fig. 4. Rabbit. The border membrane between the epithelial cells is smooth and the terminal bar (T) is observed in a part of the membrane. ×23,000.

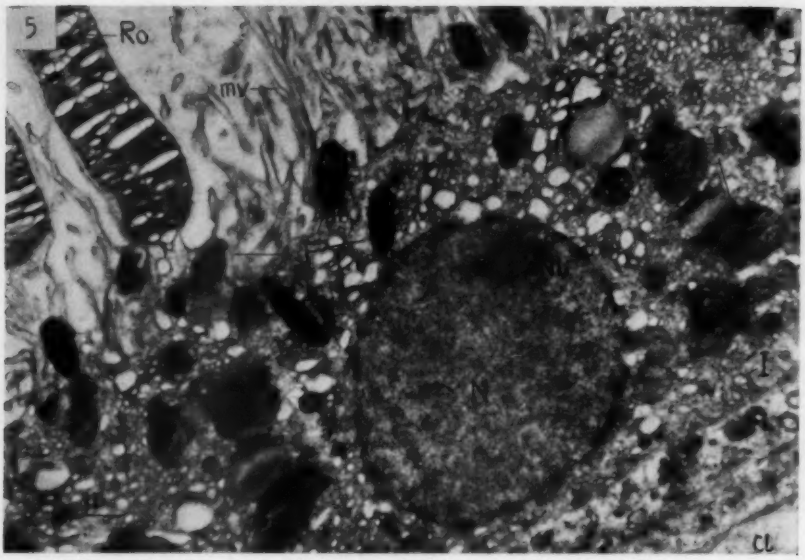

Fig. 5. Human. Fuscin granule (F) is recognized in a part of microvilli-like processes and this part shows slight swelling. Fuscin granules are classified into the spindle shaped group and the oval or round shaped group. Also, possibly the transitional form seems to exist. × 10,000.

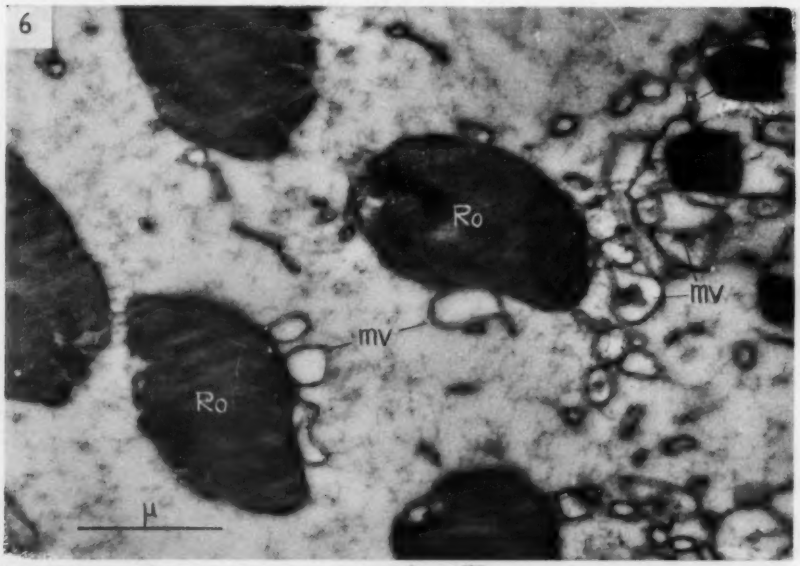

Fig. 6. Human. The horizontal section of the rod's outer segment: The microvilli-like prosesses are in direct contact with the lateral side of the rod's outer segment, but the transitional form directly to the visual cell-membrane can not be recognized. × 25,000.

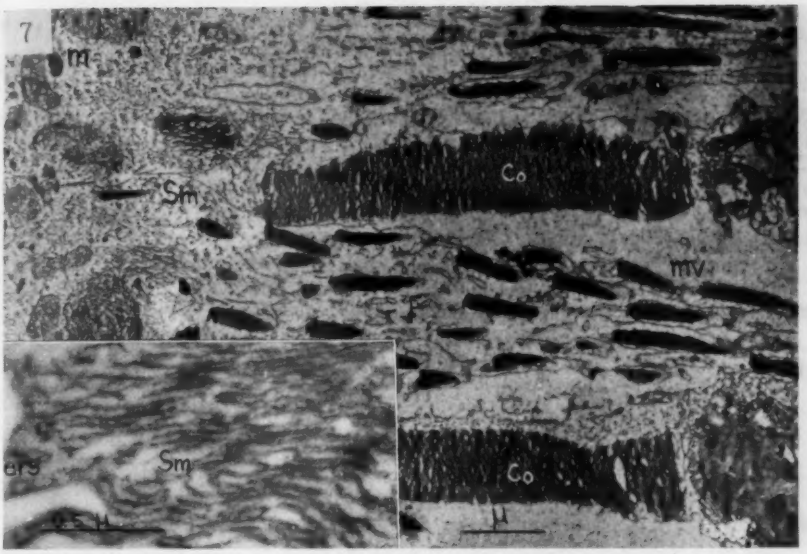

Fig. 7. Uroloncha striata. At half level of the epithelial cell, lamellar structures of various shapes are observed (Sm) and most of them are generally arranged perpendicular to the basal cell border. On the cell surface, the microvilli (mv) containing fuscin granules (F) are observed. The insertion in Fig. 7 is the lamellar structure under high magnification. × 13,000. Inset, × 53,000.

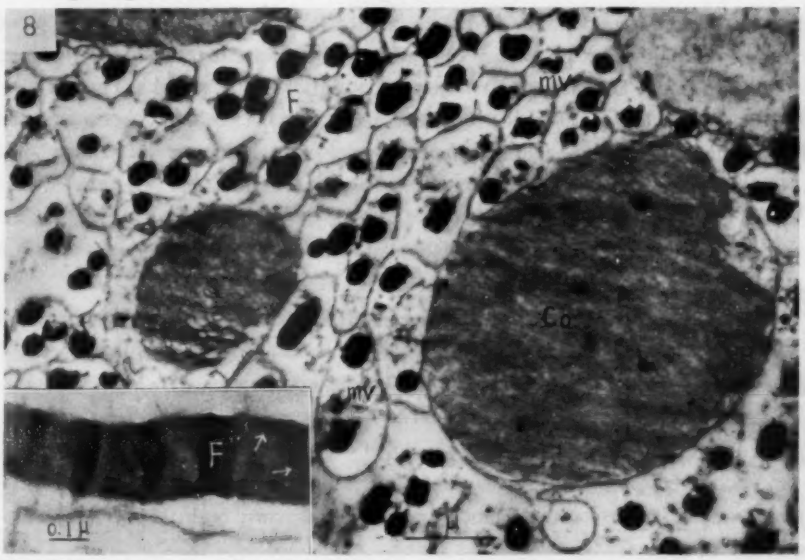

Fig. 8. Uroloncha striata. The horizontal section of the cone outer segment: Fuscin granules are observed in microvilli. The insertion in Fig. 8 shows the fuscin granule under high magnification. As inner composition, many fine tubular structures (as pointed by the arrow) are observed in the form of a reticular structure. × 16,000. Inset, × 60,000.

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EXPERIMENTAL STUDIES ON THE ALLERGY OF THE BRAIN WITH SPECIAL REFERENCE TO THE MECHANISM AND PATHOGENESIS OF RETROBULBAR NEURITIS REPORT I. SEROLOGICAL AND HISTOLOGICAL STUDIES

Syuji SUGAWARA

Department of Ophthalmology, Faculty of Medicine, Tohoku University

As pathogenic factors of retrobulbar neuritis, the so-called demyelinating diseases have been long in the foreground in the Western countries and the close relation between the former and multiple sclerosis among the latter in particular has been offering a center of interest to clinicians and neuropathologists, but in Japan, the occurrence of multiple sclerosis has been denied till quite recently, so that the relation between retrobulbar neuritis and multiple sclerosis has been not only neglected but even flatly denied.

In the latest years, however, some case reports of multiple sclerosis have begun to appear from clinics of internal medicine and neurology in Japan, so that this disease has come to be taken up as a problem and the relation between it and retrobulbar neuritis has become the theme of interest among the Japanese researchers, too.

It has become confirmed that retrobulbar neuritis observed in the patients of demyelinating diseases is not an accidental complication but that it is due to demyelinative lesions taking place with the optic nerves essentially consisting of cerebral white substance as the field of occurrence. Among the etiological propositions on it, it was the theory of allergy that has drawn our particular interest.

From such a point of view, we studied the changes occasioned in rabbits by injection with emulsions of heterologous, homologous and their own brain tissues, from serological and pathological angles.

Experimental Methods

Rabbits were injected with fresh beef and rabbit's brain and spinal cord tissues

suspended in adjuvants prepared in general compliance with FREUND's method, intradermally in their foot soles. In autoinjection, the frontal lobe was excised, similarly suspended in the adjuvants and injected into the sole of the same rabbit. One cc of the injected suspension contained 200 mg of brain or spinal cord tissue, 0.2 cc of dehydrated lanolin, 0.2-0.4 cc of liquid paraffin and 0.5 mg of components of dead tuberculosis bacilli. The injection was applied only once per animal in the dosis of 0.5 cc each.

After the injection, the antibody value was meassured every week. Following the method of SACHS, 0.25 cc each of serum, antigen, complement and hemolysin was used. As antigen, aqueous emulsions and alcohol extracts of various splanchnic organs besides the brain and the spinal cord were used. The results were evaluated by the grade of turbidity and marked as +4, +3, +2, +1 and 0, respectively.

Results

After the lapse of a certain latent period following the injection of brain emulsion, the rabbits begin to suffer from paralysis in their limbs and mostly die in 5-6 days. The latent period ranged from 13 to 18 days in the animals injected with homologous and heterologous brain emulsions and 23 to 26 days in the animals injected with emulsion of their own brain, the period being prolonged about 10 days in the latter. Besides, in 3 cases the latent period extended over 2 months and then the paralysis set in slowly, showing a chronic type, while it was of higher interest that in 2 cases, which showed acute and vehement onset of paralysis and were anticipated to die before long, began to recover in 2-4 weeks and were finally cured to normal health.

The rate of morbidity was 60% (6/10) among the animals with heterologous injection, 40% (4/10) among those wich homologous injection and 13.3% (2/15) among those injected with their own brain emulsion.

In their eyes, no severe visual trouble or anomaly in the pupil was encountered with. In the fundus, though slight turbidity in the papilla was observe in some cases, no very perceptible anomaly was detected, while in about half of the cases, the veins were found slightly dilatated and tortuous.

1) Complement Fixation Test Results

When aqueous emulsions of various organs had been applied as antigen. The results with normal rabbit serum were as shown in Tabl. 1., showing that the reaction was moderate with the brain, the testis and the kidney, but very slightly with the liver and the spleen. The results after injection of beef brain plus adjuvants were as shown in Tabl. 2. Upon comparison of these two sets of results, we find, that when brain emulsion had been applied, the antibody value to the brain was raised, but the values to other organs were scarcely affected.

When the adjuvants containing no brain tissue had been injected, it was ascertained, that no new antibody was produced in the serum, quite as in the case with normal rabbits.

Table I. Summary of Results of Complement Fixation Tests Conducted with Sera of Normal Rabbits.

Antigen	Dilution of serum						
	1:5	1:10	1:7	1:5	1:50	1:83	1:125
Brain	+ 2	+ 1	0	0	0	0	0
Testis	+ 4	+ 3	+ 1	0	0	0	0
Kidney	+ 4	+ 2	+ 1	0	0	0	0
Liver	+ 1	0	0	0	0	0	0
Spleen	+ 1	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0
Lung	0	0	0	0	0	0	0

Antigen: aqueous emulsions
Dilution of antigen: 1:60

Table II. Summary of Results of Complement Fixation Tests Conducted with Sera of Rabbits Immunized with Heterologous Brain Plus Adjuvants.

Antigen	Dilution of serum						
	1:5	1:10	1:17	1:25	1:50	1:83	1:125
Brain	+ 4	+ 4	+ 2	+ 1	0	0	0
Testis	+ 4	+ 2	+ 1	0	0	0	0
Kidney	+ 4	+ 3	+ 2	+ 1	0	0	0
Liver	+ 1	0	0	0	0	0	0
Spleen	0	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0
Lung	0	0	0	0	0	0	0

When aqueous emulsions were used as antigens in the tests, lack of organ specificity is evident.
Dilution of antigen: 1:60

When the natural antibody had been destroyed. When serum is warmed at 65°C for 45 min. according to KIDD's method, the non-specific antibodies are exterminated and any sample of normal rabbit serum thus treated loses all capacity to react with any antigen, but the serum sampled from rabbits injected with brain emulsion reacts quite specifically with brain antigen alone, even after such a treatment (Tab. 3).

After use of alcohol extracts of various organs as antigen. As shown in Tab. 4, the reaction was found specific to the brain and the importance of lipoids in the antigen-antibody reaction in such experiments was ascertained.

Table III. Summary of Results of Complement Fixation Tests Conducted with Sera of Rabbits Immunized with Heterologous Brain plus Adjuvants.

Antigen	Dilution of Serum						
	1:5	1:10	1:17	1:25	1:50	1:83	1:125
Brain	+ 4	+ 4	+ 2	0	0	0	0
Testis	0	0	0	0	0	0	0
Kidney	0	0	0	0	0	0	0
Liver	0	0	0	0	0	0	0
Spleen	0	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0
Lung	0	0	0	0	0	0	0

After destruction of natural antibody, the organ specificity is evident even upon use of aqueous emulsions.
Dilution of antigen: 1:60.

Table IV. Summary of Results of Complement Fixation Tests Conducted with Sera of Rabbits Immunized with Heterologous Brain plus Adjuvants.

Antigen	Dilution of serum						
	1:5	1:10	1:17	1:25	1:50	1:83	1:125
Brain	+ 4	+ 4	+ 4	+ 2	+ 1	0	0
Testis	0	0	0	0	0	0	0
Kidney	0	0	0	0	0	0	0
Liver	0	0	0	0	0	0	0
Spleen	0	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0
Lung	0	0	0	0	0	0	0
Skin	0	0	0	0	0	0	0

When alcohol extracts were used as antigens in the tests, organ specificity is evident.
Dilution of antigen: 1:60.

Absorption test results. Through absorption tests with extracts of various organs, the specificity of the reaction of serum rabbits injected with brain emulsion to the brain was further confirmed.

After use of different parts of the central nervous system as antigen. The complement fixation reaction after use of the cerebral white and gray matters, the medulla, the spinal cord and the optic nerve as antigen, as shown in Tab. 5, showed little difference, while no difference was found in the antigenicity of the cerebra of guinea-pigs and rabbits,

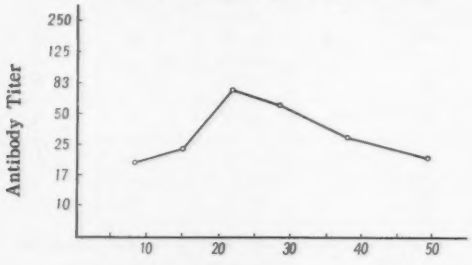
showing the absence of species specificity. It is seen that the optic nerves react as strongly as the central nervous system indicates, that pathological changes possible in the central nervous system can come forth also in the optic nerves.

Table V. Summary of Results of Complement Fixation Tests Conducted with Sera of Rabbits Immunized with Heterologous Brain plus Adjuvants.

Antigen		Dilution of serum						
		1:5	1:10	1:17	1:25	1:50	1:83	1:125
Beef brain		+ 4	+ 4	+ 2	+ 1	0	0	0
Guinea-pig brain		+ 4	+ 4	+ 3	+ 2	0	0	0
Rabbit brain	white matter	+ 4	+ 4	+ 3	+ 1	0	0	0
	grey matter	+ 4	+ 4	+ 3	+ 2	+ 1	0	0
Rabbit medulla		+ 4	+ 4	+ 3	+ 1	0	0	0
〃 cerebellum		+ 4	+ 4	+ 3	0	0	0	0
〃 cord		+ 4	+ 4	+ 4	+ 3	+ 1	0	0
〃 optic nerve		+ 4	+ 4	+ 2	+ 1	0	0	0

When alcoholic extracts of the cerebra of guinea-pigs and rabbits and various other parts of the central nervous system of rabbits were used as antigen, the antibody values are nearly equal. Note that the antigen antibody reaction is strong also when alcoholic extracts of the optic nerves is administered.

Fig. 1. Change in antibody value following injection of heterologous brain plus adjuvants.



The antibody value rises to the maximum around the 3rd week.

Change in the antibody values after brain emulsion injection. The antibodies were produced first in one week after injection with brain emulsion plus adjuvants, their value rose gradually to reach the maximum in three weeks and dropped off slowly there after. That is to say, the antibody value rose to the maximum at the time when the animals fell sick (Fig. 1). No difference in the antibody values was found in the diseased and the non-diseased animals.

In addition, the antibody values differed in descending order in the animals after

heterologous, homologous and own-brain injection. It is of interest that this order is in coincidence with the order of the morbidity rate.

2) Results of Pathohistological Examination

Relation between clinical symptoms and pathological picture. In general, in the acute cases where the disease fell on abruptly and death came on in a few days, the changes in the tissues of the central nervous system were severe and wide-spread, but in a few of such cases we could find only unexpectedly light pathohistological changes, while in a few of such cases of not so severe clinical symptoms, we were surprised to observe prominent histological changes. Therefore, a strict parallelism between the clinical symptoms and the pathological changes cannot be established, but at least a general parallelism must be admitted here.

Characteristics of the histological changes of the central nervous system. The patho histological findings we observed in the spinal cords, the brains and the optic nerves, and their characteristics may be summarized as follows.

(i) The lesions were always found around the small blood vessels, especially, the veinlets, scarcely any lesions being found independent of the blood vessels. Most of such lesions were seen elliptical, but as they were formed encircling the blood vessels, they are probably cylindrical in actual form.

(ii) The lesions showed infiltration of lymphocytes, monocytes, neutrophil leucocytes, histiocytes and plasmocytes and later on, microglial cells also came in.

(iii) In the same individual animal, some difference was found in the freshness of the lesions, which showed the tendency of increasing in area with the lapse of time and sometimes more than one lesion was found fused together in one.

(iv) Demyelination was nearly always found accompanying the cell infiltration, and though also the axis cylinders were found demolished in the loci of strong pathotic changes, they were always affected more lightly than the myelin sheaths. There was no demyelination focus free of cell infiltration, but in some lesions of a very early stage, there were some with cell infiltration nearly free of demyelination. It follows that demyelination represents a secondary change process.

(v) Cell infiltration was found sometimes in the blood vessel walls themselves, and sometimes the lesions were accompanied by bleeding or thrombi, but not at all frequently.

(vi) The lesions are formed in any part of the central nervous tissues, but over whelmingly more frequently in the white than in the gray substance.

(vii) As extension of the changes within the central nervous tissues, inflammation came forth in the optic nerve sheaths and the meninges and cell infiltration was observed in some cases, but in some cases findings of neurothecitis of the optic nerves or of meningitis were observed, showing the picture as if inflammation had spread thence to the optic nerves or the brain and the spinal cord.

(viii) The changes tended to be the severest in the animals after injection of heterologous brain emulsion and the mildest in those injected with emulsion of their own brain.

Characteristics of the changes in the central nervous system and the optic nerves. The most typical changes were frequent in the spinal cord and especially so in its thoracic segments. The changes sometimes spread into the gray matter, but even in such cases they were always originating from the white matter. Leptomeningitis and arachnitis were also observed in a few cases. Scarcely any change was found in the spinal nerve roots.

In the brain, the changes occurred preferably in the white matter adjacent to the ventricle, where the lesions tended to be severer and larger than in the other parts of the central nervous system. On rare occasions, inflammation occurred in the pia mater or the choroid plexus. The cerebral cortex, the cerebellum, the pons and the medulla was rarely but the brain stem slightly more often affected.

A pathological picture of papillitis was found in 2 cases. Cell infiltration around the small blood vessels and demyelination accompanying it were the main symptoms, slight turbidity and dilatation and tortuosity of the blood vessels being also observed ophthalmoscopically in the papilla. In the optic nerve trunk, sometimes lesions were formed solitarily in the parenchyma, but they were most frequent in the parts neighboring the optic nerve sheaths, which were sometimes affected themselves. In the chiasm too, the changes were most frequent in the vicinity of the nerve sheaths. In the optic nerves, it was characteristic that microglia cells came in earlier and more abundantly than in the brain and the spinal cord.

Prominent change was never found in the choroid, the iris, the ciliary body and the sclera in any case.

Discussion

Many studies on experimental encephalomyelitis have been reported, in particular, in U.S.A., but owing to the reason cited above, few reports have appeared on the subject in Japan. The opinion, that the changes observable in such experiments are due to brain allergy has come into wide acceptance. Therefore, the present author made simultaneous study of the antibodies and the pathology in such experiments, and experiments with rabbits injected with homologous, heterologous and their own brain emulsions, at the same time aiming at examining the results in correlation with retrobulbar neuritis.

One of the reasons why some researchers have objected to the allergy theory on the mechanism of occurrence of experimental encephalomyelitis lies in the observation that the antibody production is unrelated to the occurrence of the disease, according to their opinion. In my experiments, however, it has been clarified that the two are correlated to some extent, since the antibody value attains the maximum level in 2-4 weeks after the injection of antigen emulsion, when the occurrence of the disease is most frequent, and there is a general parallelism observable between the morbidity rates and the antibody values in three groups of animals. From the facts that the antibodies are entirely specific to the brain and that the lesions are in the largest majority of the cases formed in the white matter of the central nervous system including the optic nerves, it may be deduced that the myelin sheaths play a leading role in the occurrence of this disease.

The break-out of disease in some and non-occurrence in others of the animals, despite production of antibodies to the same level may be interpreted as due to a difference in the endogenous susceptibility among the animals. The existence of lesions found in some cases not afflicted by the disease may be probably due to the mildness or smallness of such lesions, or the lesser importance of their loci in the central nervous system.

The pathological picture here is that of the so-called perivenous encephalomyelitis and is quite similar to that of paralysis after antirabies vaccination. It may appear rather different from the pathological picture of multiple sclerosis, but we must notice, that, while in animal experiments we are studying the picture of an acute stage, in human autopsied cases of multiple sclerosis the picture of the chronic stage is apparent, so a direct comparison of the two pictures may lead to misunderstanding. Consequently, when the findings in the lesions in the experimental animals are studied in comparison with the fresher lesions in human multiple sclerosis cases, we may see the similarity between them.

As pathogenic factors of retrobulbar neuritis have been many items have been enumerated but in Japan sinusitis has been too onesidedly overemphasized. In reaction to such an overemphasis, unique theories citing opticochiasmic arachnoiditis and beri-beri have been propounded in Japan. It is of course quite impossible to deny all these as pathogenic factors, but that these diseases are much less frequent than estimated in the past has been already ascertained in my previous study on clinical cases. The present experiments of mine have been undertaken with the aim of elucidating the pathosis of retrobulbar neuritis belonging to the category of demyelinative diseases represented by multiple sclerosis, particularly from the stand-point of allergy, abreast with clinical studies of retrobulbar neuritis.

As stated above, the mechanism of occurrence of experimentally induced anomalies in the optic nerves is quite identical with that of anomalies in the white matter of the brain and the spinal cord and the pathology of such anomalies in the nervous tissues may be most rationally explained by citing allergic reaction in the brain.

Conclusion

Aiming at clarifying the pathogenesis, the mechanism and the actual changes in retrobulbar neuritis due to demyelinating diseases which represent multiple sclerosis and other undetected causes, serological and pathological studies were performed. Using rabbits, the emulsion of heterologous, homologous or their own brain tissue was injected.

The rabbits fell into palsy of extremities about three weeks after. The paralysis originated in the white matter. In all the cases, a specific antibody to the brain was produced. It was also specific to the optic nerve. In each group, correlation was proved between the antibody value and the rate of paralysis. The antibody contributes a great deal to produce paralysis because paralysis appears when the antibody value reaches the maximum.

The changes in the optic nerve were similar to that of the other parts of the central nervous system. The main change consisted in demyelination with perivascular inflamma-

tory changes. The mechanism may be rationally explained by the theory of brain allergy.

(Published in Acta Ophthalm. Jap. 62:1506, 1958 in Japanese.)

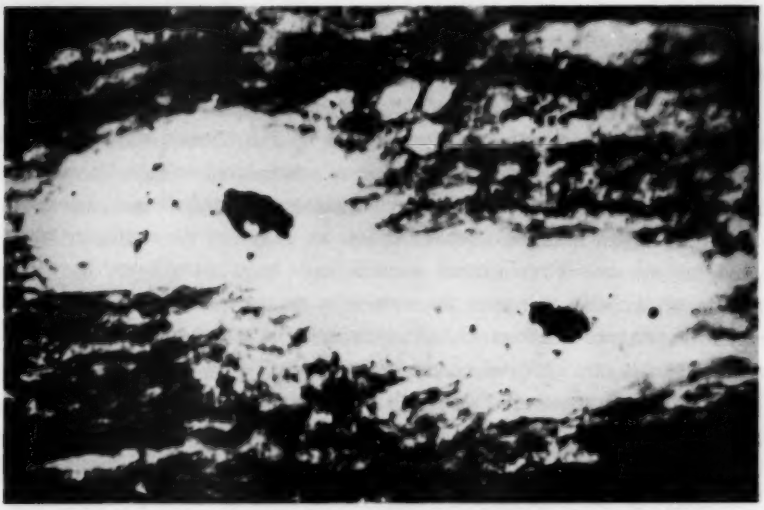


Fig. 2. Prominent fusion of demyelinated lesions in the spinal cord. MALLORY's myelin sheath staining.

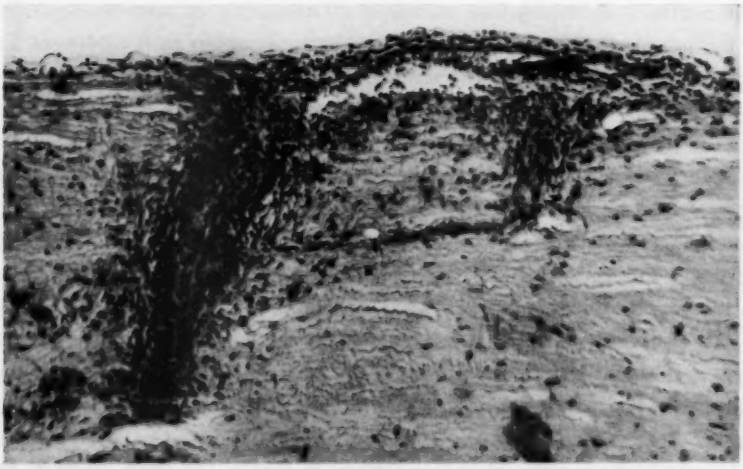


Fig. 3. Isolated lesion in the spinal cord in connection with a blood vessel from the pia mater, suggesting vascular dissemination of the lesion. H-E staining.

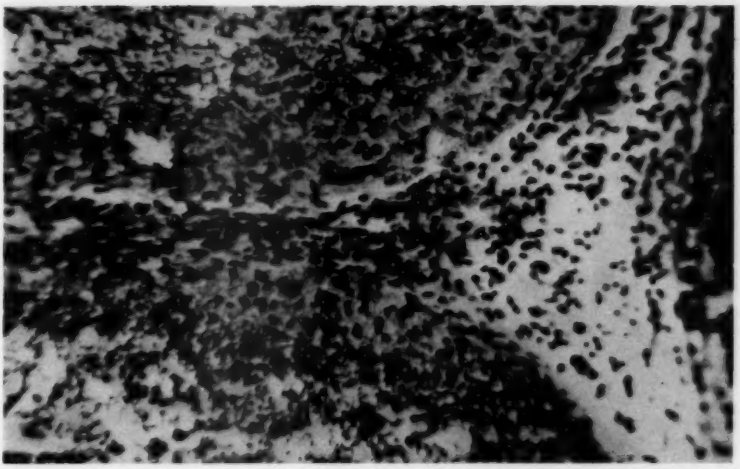


Fig. 4. Cell infiltration spreading from the arachnoiditis focus into the spinal cord and demyelination in the part. KULTSHITZKY's myelin sheath staining.

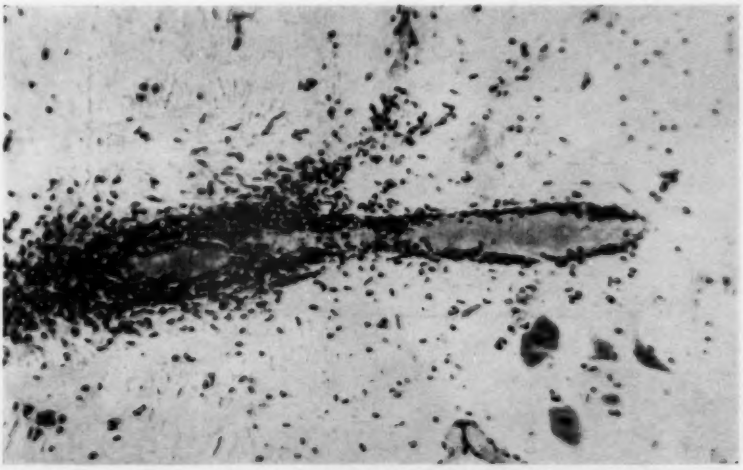


Fig. 5. A prominent perivascular cell infiltration found weakening abruptly upon reaching the gray matter. H-E staining.

Fig. 6. Myelin sheaths meandering crooked, vacuolated, swollen or rosary-form in the loci of demyelination and finally collapsed and lost. KULTSHITZKY's mydlin sheath staining.

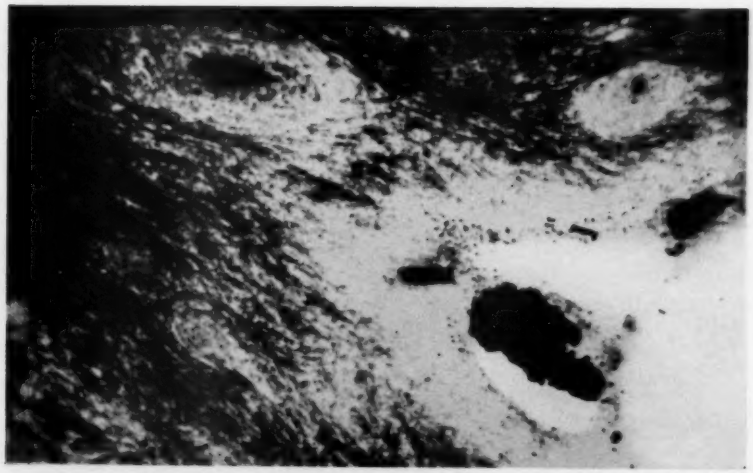


Fig. 7. Perivascular demyelination in whith matter adjacent to the ventricle. KULTSHITZKY's myelin sheath staining.

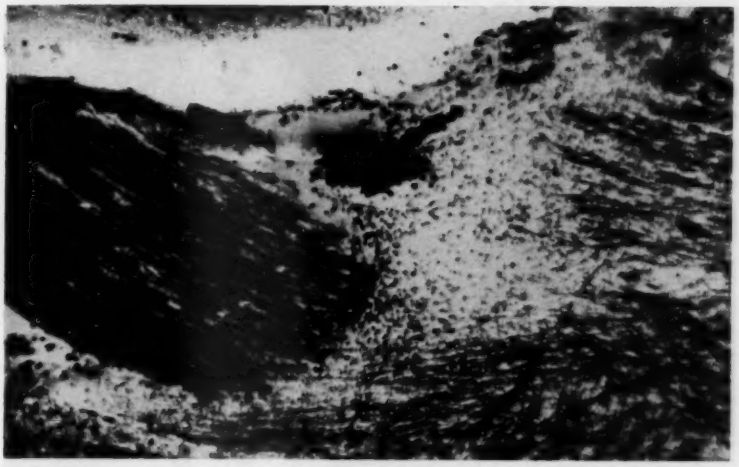


Fig. 8. Showing that perivascular cell infiltration and demyelination do not spread into peripheral nerves. KULTSHITZKY's myelin sheath staining.

Fig. 9. Cell infiltration in the papilla. H-E staining.

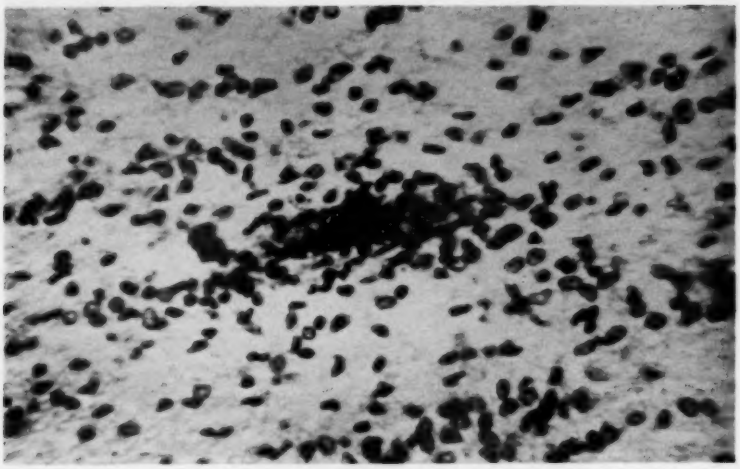


Fig. 10. Many glial cells appear at a comparatively early stage, in the perivascular cell infiltration in the optic nerve parenchyma. H-E staining.

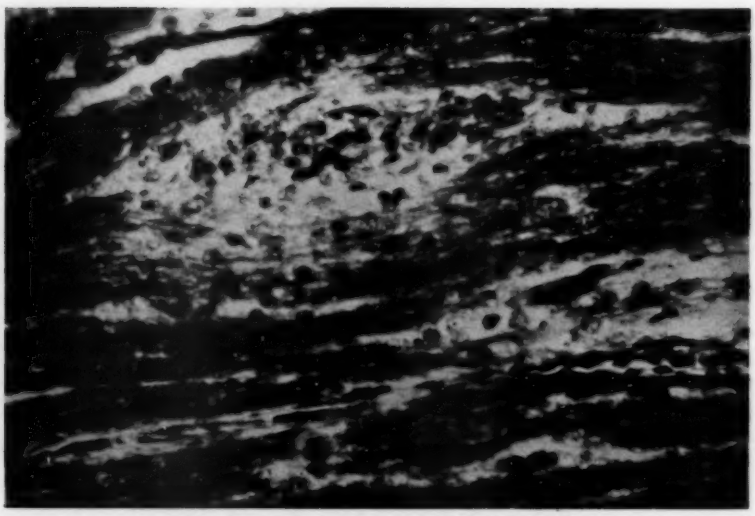


Fig. 11. Ditto, under KULTSHITZKY's staining.

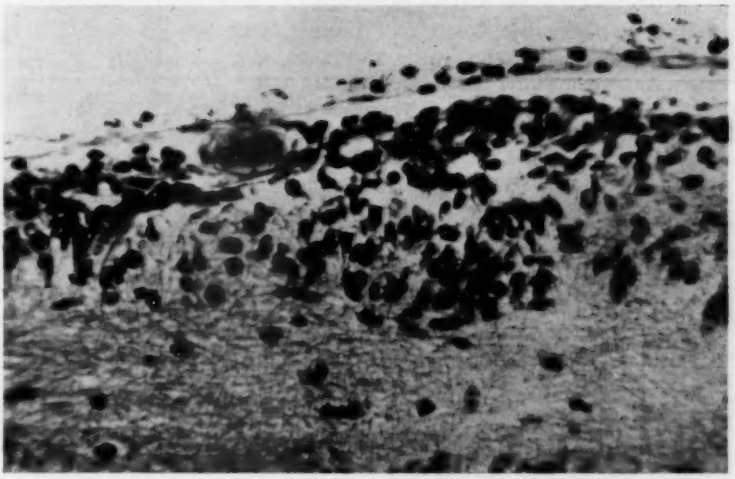


Fig. 12. Perivascular cell infiltration beneath optic nerve sheath, where too glial cells are found in an early stage. H-E staining.

Fig. 13. The myelin sheaths still nearly free from colapse in the earliest stage of perivascular cell infiltration in the optic nerve. KULTSHITZKY's myelin sheath staining.

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HISTOCHEMICAL STUDIES IN FRESH PIGMENT EPITHELIUM
II. ISOCITRIC DEHYDROGENASE AND CYTOCHROME OXYDASE

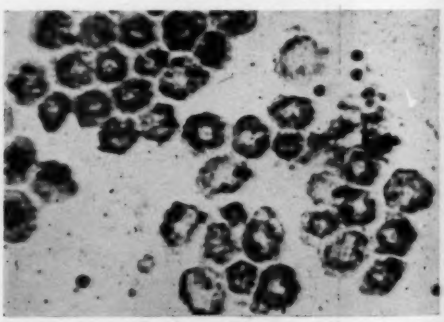
Takashi MIZUKAWA, Masakazu IGA, Shogo KUBO
and Morikata YAMAMOTO

Department of Ophthalmology, Osaka University Medical School

As they described in the previous paper[1], the authors succeeded in preparing fresh specimen of the pigment epithelium. This enabled them to study histochemically the pigment epithelium under natural conditions, avoiding the effects of fixatives and the following destruction of cells (Fig. 1). By this histochemical method, they demonstrated the existence of succinic and malic dehydrogenases in the pigment epithelium.

Fig. 1. (Uyama et al.). Fresh bovine pigment epithelium.

Fig. 2. (Uyama et al.). Succinic dehydrogenase—TTC.



At the same time the authors studied the cat retina which has the same tapetum as the bovine retina, in order to locate mitochondria by vital fixation. They learned that the activities of succinic and malic dehydrogenases in the bovine retina were found at the same places in the protoplasm of the pigment epithelium as the mitochondria in the cat

retina (Figs. 2,3 and 4). So they guessed that these enzymes and mitochondria are closely related and the process succinate⇒fumarate⇒malate⇒oxaloacetate would occur in the protoplasm of the pigment epithelium.

Fig. 3. (Uyama etal). Succinic dehydrogenase—NT.

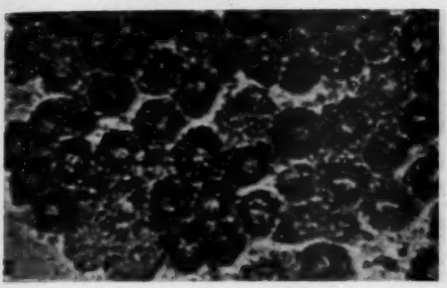
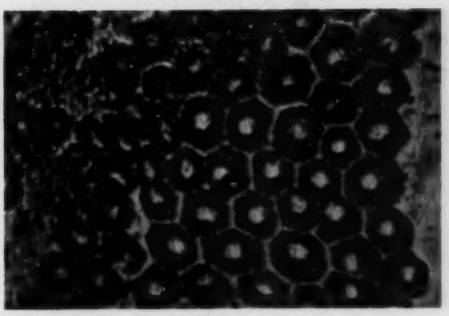


Fig. 4. (Uyama et al). Malic dehydrogenase—NT.



In the present paper, the authors will report that they found the dehydrogenases for citrate and isocitrate in the pigment epithelium of the bovine retina. Futhermore, they will also report that they demonstrated the existence of cytochrome oxydase.

Material and Method

The eye of freshly killed cattle were enucleated and bisected equatorially with a razor blade and scissors into anterior and posterior segments. The retina was removed from each posterior half and the pigment epithelia were mounted on a microscopic slide smeared with a safety razor, as stated in the previous paper (Medical Journal of Osaka University, Vol. 7, No. 3, 1956).

Staining

For the observation of iso-citric dehydrogenase, sodium iso-citrate was used in place of sodium succinate in the case of Ishikawa's method for succinic dehydrogenase. Both 2-3-5-triphenyltetrazolium chloride (TTC) and neotetrazolium chloride (NT) were used as the acceptors of hydrogen. Also the reducing action for citrate was tested by the same method. Gräff's method was used for cytochrome oxydase.

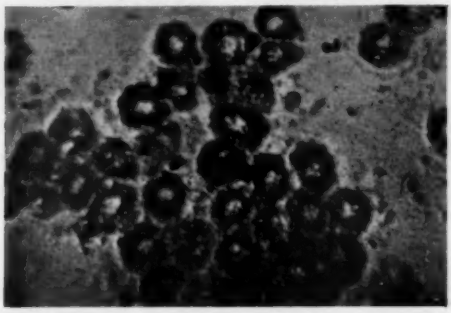
In the previous experiment, the pigment epithelia mounted on a microscopic slide were incubated in the stain solution at 37°C for an hour aerobically in the open vessels. But, at this time, besides mounted pigment epithelia blocks of pigment epithelia were also tested. After being incubated in the stain solution, the blocks were mounted on a microscopic slide. Better results were obtained by this method. As to this method Seligman and Rutenburg[2] remarked that sometimes other dehydrogenases acting on endogenous substrates are responsible for this phenomenon. So the results will be discussed in detail in this paper.

Experiment

It is hard to mount the horizontal sections of the pigment epithelium of the paraffin embedded retina but, by the above-mentioned smearing technique, hexagonal cells of the pigment epithelia could readily be obtained. Fig. 1 shows the pigment epithelia juxtaposed like paving stones.

Iso-citric dehydrogenase. Reddish color is observed in iso-citric dehydrogenase positive specimens with the present method and this coloring is seen intracellularly except the nucleus. Many reddish granules were scattered throughout the protoplasm (Fig. 5). These granules varied in size. Some resembled mitochondria in shape and distribution. The nucleus did not exhibit enzymatic activity.

Fig. 5. Iso-citric dehydrogenase—NT.



	1	2	3	4
Pigment epithelium	+	+	+	+
0.1M dl-Sodium iso-citrate	+	+	−	−
0.05% NT or 0.05% TTC	+	−	+	−
0.1M Phosphate buffer (pH 7.4)	+	+	+	+
Result	Stained	Not stained	Not stained	Not stained

Fig. 6. Reducing action for citrate—NT.

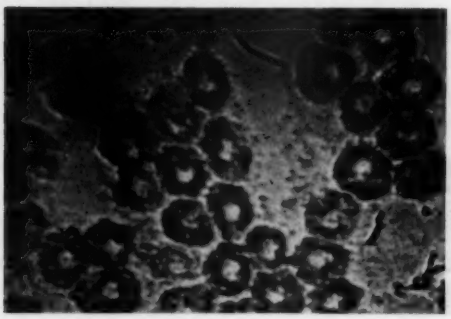
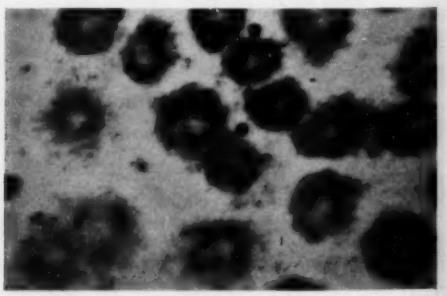


Fig. 7. Cytochrome oxydase.



When TTC and NT were used as the acceptors of hydrogen, they showed no differences in coloring in the present experiments.

Reducing action for citrate. The authors used the word "reducing action for citrate", because the existence of the enzyme "citric dehydrogenase", so-called by Lison, is not clear. In this case, similar reddish granules as in the case of iso-citric dehydrogenase were seen scattered in the protoplasm outside the nucleus (Fig. 6). These granules resembled the iso-citric dehydrogenase positive granules in size, shape and distribution.

Cytochrome oxydase. The cytochrome oxydase activity was present in the protoplasm of the pigment epithelium. The pattern of distribution of the deep-blue granules was similar to that of above-mentioned reddish granules but deep-blue ones were scattered more densely. No enzymatic activity was demonstrated in the nucleus of the pigment epithelium (Fig. 7).

Sodium azide (0.005 M) caused almost complete inhibition of this enzyme as was stated by Moog.

Discussion

Succinic dehydrogenase plays a great role in most living cells and forms a link in Krebs' cycle associated with oxidation of lipids, carbohydrates and protein. Histochemical demonstrations of the succinic dehydrogenase activity have been described by many workers. They used fresh frozen sections. Using blue tetrazolium as the acceptor of hydrogen, Seligman and Rutenburg[2] found that very thin sections (8μ) failed to stain altogether, whereas thick sections (30-40μ) uniformly showed a deep-blue stain, and thin sections of some tissues which had a high order of enzymatic activity were stained lightly. In the present experiment fresh smear preparate had the thickness under 7-8μ, but good results were obtained. Moreover, in the absence of succinate, it was not stained. It was also the case when blocks of the retina were used. Therefore, in the present experiments, other dehydrogenases acting on endogenous substrates were not responsible for this reaction. So it can be concluded that the reaction is specific for succinic dehydrogenase, by the absence of staining, when the substrate was omitted from the incubation medium and by inhibition of the reaction with malonic acid (0.5 M). As for succinic dehydrogenase, its activity in the retina was studied biochemically by Tahara[3], Nitta[4], Iguchi[5], Sakai[6], and histochemically by Akiya[7], Kojima[8], Wislocki and others[9]. Its activity in the pigment epithelium was studied by the authors, which was reported in the previous paper[1].

As for cytochrome oxydase that combines oxygen with the hydrogen that is taken out of succinate and carried to cytochrome c by succinic dehydrogenase, Akiya studied in detail, using the retina of guinea pigs. He demonstrated blue granules in the visual cells, especially in the outer segment, and in the outer and inner reticular layers, nerve fiber layer, ganglion cell layer and the pigment epithelium. The authors demonstrated blue granules in the protoplasm of the fresh bovine pigment epithelium, and found that 0.005 M

sodium azide inhibits the coloring. Therefore, it is considered that the blue granules show the places where the activity of cytochrome oxydase exists.

As it was reported in the previous paper[1], the authors demonstrated the activity of malic dehydrogenase in the pigment epithelium. This enzyme causes the reaction malate⇌oxaloacetate, but acts more strongly to cause the reaction in (→)-direction than to cause it in (←)-direction, and the authors succeeded in detecting the activity of malic dehydrogenase by adding semicarbazide to this incubation medium.

The word "citric dehydrogenase" appears in Lison's book, but the existence of this enzyme is doubtful. The authors too recognized the coloring when citrate was used as the substrate. But they prefer to consider, that this coloring occurred by iso-citric dehydrogenase after citrate had been converted into iso-citrate. The authors used isocitrate as the substrate in order to establish the existence of iso-citric dehydrogenase and recognized the coloring in the protoplasm of the pigment epithelium.

Summary

1. Using the fresh pigment epithelium of the bovine retina, the auhtors found the activities of iso-citric dehydrogenase in the protoplasm, in addition to the previous discovery of the activities of succinic and malic dehydrogenases.

2. The activity of cytochrome oxydase was apparent in the protoplasm of the pigment epithelium.

3. From the above findings, it is suggested that the process succinate⇌fumarate⇌malate⇌oxaloacetate⇌citrate⇌cis-aconitate⇌iso-citrate⇌oxalosuccinate would occur in the protoplasm of the pigment epithelium.

(Published in Acta Ophthalm. Jap. 62:930, 1958 in Japanese.)

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EXPERIMENTAL STUDIES ON THE AUTONOMIC REGULATING FUNCTION OF INTRAOCULAR PRESSURE THROUGH SYMPATHETIC NERVES

Takeshi SUZUKI

Dept. of Ophth., Okayama University Medical School

It is quite imaginable, that there is an autonomic regulation function for intraocular pressure, just as in other autonomic function in the body and that it keeps the intraocular

pressure in the normal state according to the changes of the outer environment. The author is trying to certify this fact and is going to pursue further what role the sympathetic nerve is playing for this function. The author observed the change that came on the intraocular pressure under the slight pressure given to the eyeball after mechanically interfering with the tract of the sympathetic nerve to the eyeball, so that he may be also able to investigate some influence of the autonomic nervous system on glaucoma, and is trying to find its cause.

Experiment I: The intraocular pressure and the blood pressure under the compression of the eyeball.

The author investigated the change of the intraocular pressure of the eyeball under slight pressure and also that of the other eye to which no pressure was given. The author also observed the relation between the change and the blood pressure and is trying to find whether there is any function which automatically regulates the intraocular pressure or not.

The method of the experinent

As experimental animals white adult rabbits were used. Pressure was exerted on the cornea by the Bailliart's ophthalmo-dynamometer. The intraocular pressure was measured and recorded continuously on a sheet of smoked paper together with the blood pressuae of the femoral artery through a cannula. As the recording apparatus Wessely-Yada's mercury lever manometer modified by Akagi was used. The time used for pressing the eyeball was 10 minutes. During the time of measuring the intraocular pressure 10 grams of weight was loaded on the eye, while 40 grams on the opposite eye. The changes of the intraocular pressure was observed in succession from the time when the pressure was not added, being added; and after the pressure was taken off.

The result of the experinent

It was natural that the intraocular preassure in a pressed eyeball was rapidly rising or falling according to the pressure exerted or removed. It was also recognized that there was a tendency to get back to the original state which was before giving pressure, in the course of applying pressure. The intraocular pressure of the pressed eyeball fell by 0.4~1.1 mmHg during the pressure which was exerted in 5 cases without exception; and began to rise within 10 minutes after the pressure was taken off in 3 cases. In 2 cases it began to rise after 10 minutes, approaching the original pressure. The intraocular pressure of one eye, when the other eye was pressed, began to fall slowly by 0.5~2.0 mmHg a little later the pressure was added in 6 cases. After taking off the pressure, it had a tendency to rise very slowly. The alteration of intraocular pressure under pressure showed different changes than the change in the blood pressure respectively; no parallel relations between the two could be possible.

Experiment 2: Observation on the intraocular pressure and the blood pressure under the compression of the eyeball when Leriche's operation is performed.

In order to investigate how the effective influence of the sympathetic nerve imposed

upon the change of intraocular pressure in the first experiment, an experiment was carried out by cutting off the nervous network of the communicate carotide which was recognized as a tract of the ocular sympathetic nerve.

The method of experiment

As experimental animals adult white rabbits were used. Before the actual experiment the adventitia of the left carotide was peeled off about 2 cm. proximal from the bifurcation, and ethyl-alcohol (98%) was brushed on there, to complete the degeneration of the nerve fibers. In so treated animals, miosis and dilated artery from which the peeled nerves were cut off, were usually observed. For the actual experiment, only rabbits with those symptoms were used. The method of exerting pressure, the duration of pressure, the method of measuring and recording the intraocular pressure and the blood pressure, were just the same as was shown in experiment I.

The result of the experiment

After the preparatory operation, miosis of the eye of the operated side was perceived in 7 cases except 1. The intraocular pressure as measured with Shiötz's tonometer showed elevation after the operation in 4 cases out of 5. It was considered that this was caused by the interruption of the sympathetic nerve control. The intraocular pressure of of the eye of the non-operated side, when the pressure was given to the other eyeball fell by 0.4~0.5 mmHg in 4 cases, which is almost equal to the case of the eye of a normal rabbit as in experiment I; and after taking off the pressure, almost the same change of intraocular pressure as in the normal eye was seen. The intraocular pressure of the eye of the operated side showed elevation of 0~0.5 mmHg while the pressure was being added in 5 cases without exception. After the pressure was taken off, in the cases of 3 experiments out of 5, there was no alteration; in 2 cases, a slight elevation of intraocular pressure could be observed. The intraocular pressure of the eye of the operated side showed the depression of 0~3.3 mmHg even during the compression, as if it were normal, but after pressure was taken off, there was no definite tendency in all cases; in some, slight elevation; in some, slight depression and in others no change. In 10 minutes after the pressure was taken off, with only one exception, there could be seen a little elevation, comparing with the intraocular pressure before the compression. Judging from these facts, the author considers, that these are in unstable condition. The intraocular pressure of the eye of the nonoperated side showed depression during the compressions. This result was the same as of normal rabbits. In 10 minutes after the pressure was taken off, the intraocular pressure, though not greatly, showed up less than the original. We could not recognize that the intraocular pressure and the change of blood pressure were parallel with each other.

Experiment 3: Observations on the intraocular pressure and the blood pressure were under compression of the eyeball when the superior cervical sympathetic ganglion is extirpated.

In order to investigete what change comes to the autonomic regulating function of

intraocular pressure, pressure was given to an eyeball after cutting off the superior cervical ganglion, which was considered to have a close connection with the eyeball as the tract between the sympathetic nerve and the eyeball.

The method of experiment

We used a white adult rabbit in which, as preparation, the leftside sympathetic superior cervical ganglion was cut oft. The apparatus and the mode of experiment were just the same as those of experiment I.

The result of the experiment

The opposit side intraocular pressure in the case of pressing eyeball of the operated side, as was shown in the eye of the normal rabbit, was reduced 0.7~0.8 mmHg in 4 cases without exception and after taking off pressure, there appeared a tendency of coming to the original pressure.

The intraocular pressure of the operated side, when the eye of the nonoprated side was compressed, was elevated by 0~1.6 mmHg in 4 cases without exception. There was no case of falling. After the pressure was taken off, the author observed elevation in all cases. As to intraocular pressure of the compressed eye, the author could not recognize any change in 5 cases; in one case the author saw depression, and in 8 cases, elevation was observed. The author could not see an uniform tendency, but came to consider, that the tendency of depression was rare. The author observed the depression of 0.2~ 1.5 mmHg in 5 cases without any exception on the eye of the nonoperated side under pressure, and that there was the same tendency as was shown in the normal rabbit. The author could not see any special relation betweed the changes of intraocular pressure caused by pressure and the change of blood pressure measured at the same time in 8 cases without exception.

Experiment 4: Observation on the intraocular pressure and blood pressure under the compression of the eyeball when stellectomy is performed.

The experiment was carried on in order to investigate the effect of a stellate ganglion by inspecting what change was shown on autonomic regulating function of intraocular pressure when the stellate ganglion was removed, in comparison with the normal eye.

The method of experiment

We used a white adult rabbit in which, as preparation, a leftside stellectomy was performed. The apparatus and the mode of experiment were just the same as those of experiment I.

The result of the experiment

The intraocular pressure of the compressed eye under pressure began to decrease by 0.3~2.7 mmHg in all 4 cases; there was a tendency to come to the original pressure, showing slight disturbance after the pressure was taken off. The changes were just the same as in the case of a normal eye. On the contrary, in the case of the eye of the operated side, an elevation of 0~0.1 mmHg was seen in all 4 cases, and the author could not observe the depression of intraocular pressure as was shown in a normal eye,

and after taking off pressure, saw sometimes depression or elevation; yet approaching slowly the original pressure. The intraocular pressure of the eye of the nonoperated side, when the eye of the operated side was pressed, showed depression of 0.2～0.9 mmHg a little after the pressure was given in all 4 cases; and showed almost the same process as in the case of a normal rabbit. The intraocular pressure of the eye of the operated side during the time of pressing the nonoperated side, showed the elevation of 0～0.7 mmHg under pressure in all 10 cases; almost in all cases, remained constant, instead of the depression. Between the change of intraocular pressure due to compression and the change of blood pressure, we could not recognize anything parallel.

Discussion and Conclusions

It is already said by many authors that there is a close relation between the blood pressure of the whole body and intraocular pressure. Yet there are also some who deny this relationship. Kitasima and Hoshizumi investigated the relation between the age and the intraocular pressure. Toyota also examined the relation between the intraocular pressure and the pressure accompanied by hypertension, and denied the relation between the two. Akagi observed the change of intraocular pressure during irrigation with normal saline solution in the rabbit and recognized, that there was a function that regulates the intraocular pressure with no relation to the blood pressure. The author also obtained the result through these experiments that when the pressure was given the change in ocular pressure did not go parallel with the change of the body blood pressure. As for the changes of the intraocular pressure when pressed, Ogata, Sugazawa, Shōji, Akagi, Hiraoka, Hinokuma have observed it by using tonometer or manometer. The author also observed, using a normal rabbit, the suddenly increased intraocular pressure due to compressipn decreased nearly to its original state while under the compression, and that when the pressure was removed, the intraocular pressure that had been lower than that of the time before the pressure was given had the tendency of gradually recovering to the state before the pressure was gived. This was almost the same as what various authors have recognized and it can be a r eference, for the existence of autonomic regulating function of intraocular pressure just as Akagi says; and the author thinks, that the center of autonomic regulating function of intraocular pressure is stimulated by the increase or decreas of intraocular pressure due to the compression, and the impulse of recovering the normal intraocular pressure which is transmitted from the center to the eye, and thus the intraocular pressure is regulated. If we consider it in this way, the author's result that if the pressure is given on one eye, the intraocular pressure of the other eye decreases is quite comprehensible.

In other words this is the reason why we can think that there is an autonomic regulating function that regulates intraocular pressure. Many researchers report about the influence which a pressed eye gives to the other. Weekers reports about consensual reaction of intraocular pressure, and Wessely, Vom-Hofe and Suda obtained almost the

same result. Hirose, Nagata, Matsuka and Kurimoto say, that the change which occurs in the intraocular pressure of the both eyes parallel each other, though their opinions are a little different from Weeker's " reaction ophthalmotonique consensuelle ".

On the contrary, Morax and Magitot, Takazawa, Akagi and Nishimura and Yamamoto say that they have obtained the result that the increase of the intraocular pressure of one eye causes the decrease of that of the other eye. And there were many clinical cases in which a seemingly normal eye becomes to have a glaucoma symptom as is known as so-called sympathetic glaucoma when the other eye had the operation to decrease the intraocular pressure of glaucoma since the time of Graefe. So, there are many different views about the relation of the intraocular preseure between the two eyes, and the causes are not clear. The reason why these results are different should be considered in the way that the method of increasing the intraocular pressure was different, and naturally the factor of increasing the pressure was different. It is already reported by many authors since Adamük that there is close relation between intraocular pressure and the automatic nervous system. Yet the mechanism for the increase or decrease of intraocular pressure is not clear, and the attitude of the autonomic nerve system is so varied that not any definite opinion can be seen. In order to shut off mechanically the sympathetic nerve connected to the eyeball the writer did Leriche's operation, extripation of the superior sympathetic cervical ganglion and stellectomy. After these operations, changes of intraocular pressure was observed during the compression of the eye. In the case of pressing the eye of the operated side, the author could not find any depression of intraoculrr pressure when the pressure was given to the eye of the operated side as was seen in the case of a normal rabbit, but observed the depression of intraocular pressure in case of the eye of the non-operated side just as in the case of a normal rabbit; and by giving the pressure on the non-operated side, the depression of intraocular pressure in the pressed eye could be noticed, but no depression on the eye of the operated side, rather, the author even observed the tendency of rising.

If the readers support the opinions of Elwyn, Akagi etc. suggesting the probability of the center of the autonomic regulating function of intraocular pressure in diencephalon, and support Akagi's view that the change of the intraocular pressure of the eye is carried to the center of the regulation of intraocular pressure, then the sympathetic nerve may act as the centrifugal way to convey the impuls from the center to the eye to lower the intraocular pressure, acting as an autonomic regulating reflex circle to keep the intraocular pressure in a normal state, the author hopes that the readers will understand the result obtained by him.

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BLOOD-WATER CONTENT AND WATER-DRINKING TEST

Masakiyo YONEBAYASHI

Department of Ophthalmology, Kyoto Prefectural Medical University

In 1928, Schmidt found the intraocular pressure was risen by water drinking, and according to this fact, he offered the water-drinking test for glaucoma suspect. Recently, it is recognized that the water-drinking test is more useful and excellent than other provocative tests.

Concerning the mechanism of the water-drinking test, the majority of workers in this field believe that the sudden hemodilution caused by water intake lowers the osmotic pressure of the blood, and then enhances the difference in the osmotic pressure between blood and aqueous humor. Consequently, much more water would come into the aqueous from the blood, and thus the intraocular pressure would come to be increased.

This explanation is only logical, but appears hardly to be doubted. Neverthless, my present experiment consisting of measurements of both blood-water content and intraocular pressure before and after water drinking have revealed that this explanation is not always indisputable and other factors would be necessary for the explanation of effects of water drinking.

Method

Twenty-three normal subjects, and five patients who were diagnosed as chronic simple glaucoma and of whom two had been operated, were used for this experiment.

First, the intraocular pressure was measured by Schioetz's tonometer when they were in a fasting and resting state. Immediately after that the blood was drawn from the cubital median vein for the purpose of measuring of the blood-water content which was performed by means of Kuroda's method, and then they were told to drink 1,000 cc of water. After that, the intraocular pressure was measured successively every 5, 10 or 15 minutes, and at the same time the blood was drawn till 50-60 minutes after having drunk water.

Kuroda's method

Previously, a capillary tube made of glass is prepared (weight; a).

The blood of which the water content should be measured, is sucked into the capillary tube and enclosed in it by gas-flame, and the capillary tube enclosing the blood is weighed (weight; b).

Now, the top of the tube is opened again by gas-flame, and its contents are completely dryed up at 100°C for 24 hours, and then the tube with dry contents is weighed (weight ; c).

Blood-water content can be calculated as follows,

$$\frac{b-c}{b-a}\times 100\%$$

Results

In the majority of the normal eyes in this experiment, the intraocular pressure began to rise in 5-10 minutes and showed the maximum value in 20-30 minutes after water drinking, and then the intraocular pressure fell gradually to the initial value or the less. Only three eyes (two subjects) showed no rising of the intraocular pressure in the entire course. Rise in the intraocular pressure showed the range from +6.1 mmHg to−1.0 mmHg and its mean value was +2.03 mmHg.

In the glaucomatous eyes, except the operated cases, the intraocular pressure rose more remarkably after water drinking and showed the maximum value in 30-40 minutes, but only two eyes (one patient) showed a maximum in 10 minutes. After that, the raised intraocular pressure gradually decreased, but was not able to be completely restored to the initial value even in 50-60 minutes after water drinking. Accoriding to Leydhecker's standard (Rising of 10 mmHg or more in the intraocular pressure is positive), all normal subjects revealed negative results, and six of seven glaucomatous eyes except the operated cases showed positive results.

After all, the intraocular pressure gradually is increasing for 20-30 minutes after water drinking with the exception of a few normal eyes, and especially just in the first 5 minutes after water drinking, the intraocular pressure tends to increase, while not any changes of the blood-water content can be found at that time.

Variations of the blood-water content after water drinking can be classified under three different types. In Type I, the blood-water content decreases at first for about 30

Fig. 1. Type I in a normal subject.

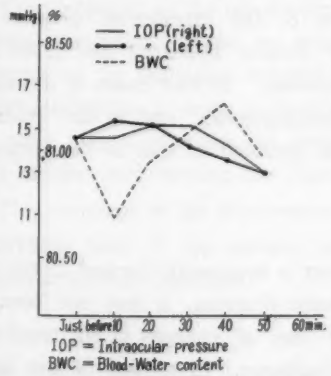


Fig. 2. Type I in a glaucomatous patient.

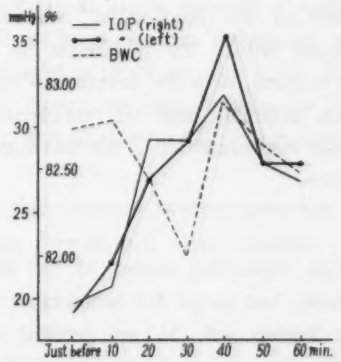
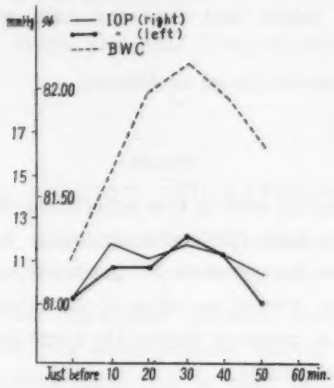


Fig. 3. Type II in a normal subject.



minutes, and then it begins to increase to the maximum amount and again begins to decrease. Twelve cases of 23 normal subjects (52%) and all the glaucomatous patients had this type (Fig. 1,2)). In Type II, variation of the blood-water content is parallel closely to that of the intraocular pressure. Seven cases of 23 normal subjects (30%) and none of the glaucomatous patients had this type (Fig. 3). In the remaining 4 normal subjects (17%), the blood-water content showed indefinite and irregular variations (Type III).

All the glaucomatous patients as well as most normal subjects showed the Type I in the variation curve of the blood-water content, that is, at first the blood was concentrated and later turned out to be diluted, but in the glaucomatous patients, the change from concentration to dilution of the blood was more intense and remarkable than in the normal subjects, and the glaucomatous patients had a more prolonged concentrated period. In the entire course, the average of the difference between maximum and minimum of the blood-water content was 0.58% in the normal, and 0.73% in the glaucomatous persons.

In 21 normal subjects except the two who did not show any distinct peak in the increase of the blood-water content, the maximum of the intraocular pressure had developed before the maximum of the blood-water content appeared and in the other eight subjects, both the maximums appeared simultaneously. In four cases of five glaucomatous patients, both the maximums developed simultaneously, and in the remaining one case the maximum of the blood-water content was preceded by that of the intraocular pressure.

Discussion

The regulating center of the blood-water content is supposedly located in the hypothalamus, but as for the function of this center in water drinking, it has not been precisely known yet. In my present experiment, the way of changes of the blood-water content after water drinking showed various kind of patterns. In a half of the normal

and all the glaucomatous patients, it was found that after water drinking the blood was concentrated at first and then diluted later. This temporary concentration of the blood just after water drinking may be caused by following factors:

1) the psychic stress under drinking a large quantity of water
2) the agony of the expansion of the stomach
3) the sudden fall of temperature of the stomach
4) the temporary decrease of temperature of the circulating blood through the absorption of cold water

These factors stimulate the center of autonomic nervous system, and produce the sympathicotonic state which gives rise to the centralization of the blood, that is, the constriction of the peripheral vessels and the influx of the circulating blood-plasma into the liver or the other internal organs. Consequently, the quantity of the circulating blood decreases, and the concentration of the blood appears. This is probably the reason why the blood is concentrated temporarily just after water drinking. Besides, I have already proved the temporary increase of the blood sugar or the blood pressure at a period synchronous with that of the blood-concentration, and therfore it seems to be sure that the sympathicotonic state may be caused temporarily just after water drinking.

Besides, many workers have shown conclusively that autonomic nervous system plays very important parts in the regulation of the intraocular pressure and the sympathicotonic state generally produces the elevation of the intraocular pressure. So, the sympathicotonic state caused by water drinking will lead to the elevation of the intraocular pressure and the concentration of the blood. After that, with the disappearance of both, the psychic agony for water drinking and the lowering action of cold water on the temperature of the stomach and the circulating blood, the parasympathicotonic state becomes temporarily predominant because of the competition between the sympathetic and the parasympathetic nerve, and then the water absorption through the stomach and intestine becomes remarkable and intense, so the transient hemodilution developes, and now it comes to take a place as a factor in the elevation of the intraocular pressure.

The variation of the blood-water content is proportional to the strength of autonomic nervous stimulation caused by water drinking and it is profoundly influenced by the difference of the individual disposition in autonomic nervous function. So, if the stage of hemoconcentration is more protracted after drinking, these cases may develop the Type I., and other cases, in which the sympathicotonic state just after water drinking disappears more rapidly, may develop the Type II.

The variation of the blood-water content in the glaucomatous cases is generally more remarkable than in the normal cases, because the former are more unstable in the autonomic nervous function.

Summary

It seems unjustified to conclude, that the elevation of the intraocular pressure is

accompanied by the increase of the blood-water content in an earlier stage at least after water-drinking test.

It is rather reasonable to believe, that the stimulation of the autonomic nervous system caused by drinking a large quantity of cold water, constitutes a primary and essential factor in the elevation of the intraocular pressure.

The pattern of the variation in the blood-water content after water drinking reveals a more unstable state in the function of autonomic nervous system in the glaucomatous patients than in the normal subjects.

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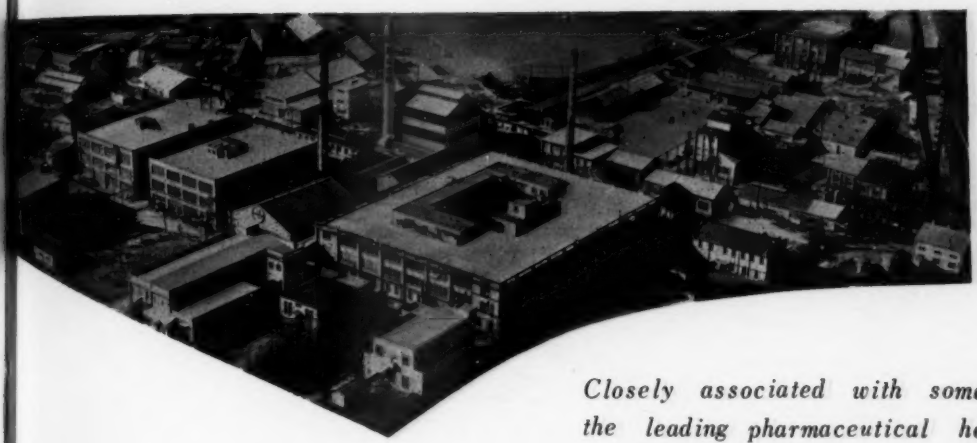

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